UNDERSTANDING THE SHEDDING PATTERN OF ANTIMICROBIAL RESISTANT *Escherichia coli* IN GOAT MEAT PRODUCTION SYSTEM: A LONGITUDINAL STUDY

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JANUARY, 2022

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Thesis submitted to the

KARNATAKA VETERINARY, ANIMAL AND FISHERIES SCIENCES UNIVERSITY

in partial fulfillment of the requirements

for the award of the degree of

MASTER OF VETERINARY SCIENCE

in

LIVESTOCK PRODUCTS TECHNOLOGY

By

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CERTIFICATE

This is to certify that the thesis entitled "***UNDERSTANDING THE SHEDDING PATTERN OF ANTIMICROBIAL RESISTANT Escherichia coli IN GOAT MEAT PRODUCTION SYSTEM: A LONGITUDINAL STUDY"*** submitted by **Mr. BHARATH, R.,** I.D. No. **MVHK 1918** in partial fulfillment of the requirements for the award of degree of **MASTER OF VETERINARY SCIENCE** in **LIVESTOCK PRODUCTS TECHNOLOGY** of the Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar is a record of bonafide research work carried out by him during the period of his study in this University, under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

Place: Bengaluru

Date:

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Affectionately dedicated to my

Family and Teachers

ACKNOWLEDGEMENT

First and foremost, I offer my prayers to ***Almighty*** *for giving me strength to endeavor and overcome the hurdles and pave my way.*

I avail this opportunity to express my deep sense of gratitude and warm regards to, ***Dr. S. Wilfred Ruban,*** *Associate Professor and Head, Department of Livestock Products Technology, Veterinary College, Bengaluru and Chairman of my Advisory Committee. He was very helpful to me throughout the course of my study and research programme. I will always remember with gratitude the innumerable ways he has influenced my life during my course.*

I am extremely grateful and indebted to ***Dr. M. K. Tandle,*** *Director of Instruction, PGS, KVAFSU, Bidar for his inspiring guidance, valuable suggestions and constant encouragement, as a member of the advisory committee.*

I am extremely grateful and indebted to ***Dr. Srikrishna Isloor,*** *Professor, Department of Veterinary Microbiology, Veterinary College, Bengaluru for his inspiring guidance, valuable suggestions and constant encouragement, as a member of the advisory committee.*

It gives me immense pleasure to convey my sincere thanks to ***Dr. Md. Mudassar Chanda,*** *Senior Scientist, ICAR-NIVEDI, Bengaluru for his valuable guidance, sincere advice and support throughout this study and for guiding in descriptive analysis as member of the advisory committee.*

I am extremely grateful and indebted to ***Dr. Kiran, M,*** *Assistant Professor, Department of Livestock Products Technology, Veterinary College, Bidar for his inspiring guidance, valuable suggestions and constant encouragement throughout the study period as a member of the advisory committee.*

I would like to place on record my sincere gratitude and thankfulness to ***Chiranjeevi Reddy and Umesh,*** *Hyadalu village, Nelamangala taluk, Bengaluru rural district for permitting me to utilize the animals and the facilities in their goat farm for my research work.*

I would like to place on record my sincere gratitude and thankfulness to the ***Department of Animal Husbandry and Veterinary Services, Government of Karnataka*** *for deputing me for the higher studies.*

My special and unforgettable thanks go to ***Dr. Sudarshan. S,*** *Assistant Professor, Department of Livestock Products Technology, Veterinary College, Bengaluru for the continuous support of my M.V.Sc study and research.*

My special thanks to ***Dr. Shivappa Nayaka, H. B,*** *Scientist (Animal Science) KVK Konehalli,* ***Dr. Shivakumar Kabla,*** *Assistant professor and Ph.D scholar at Veterinary College, Bengaluru for the continuous support in my M.V.Sc research.*

My deep sense of gratitude and profound thanks to my colleagues ***Mr. Ahlen Ig Barry, Dr. Shanthesha. K. M****,* ***Dr. Manoj, Dr. Ramya and Dr. Vinay****. I also thank Smt.* ***Sumathi*** *for her help during my research work.*

It is like a drop in the ocean of words that can never reach its mark to acknowledge infinite love, blessings, sacrifices and special gratitude to my ***Father S. Ramachandra Rao, Mother Smt. R. Manjula Bai, my Wife Smt. V.T. Geetha bai, my brother R. Keshav, my son Charvik. B. Suryavamshi and my daughter Diya Bharath*** *for their unfailing Patience, Unconditional love and affection during my entire study.*

Bengaluru

January, 2022 (***Bharath. R,)***

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LIST OF ABBREVIATIONS

%	:	Per cent
ºC	:	Degree Celsius
cm	:	Centimetre
µg	:	Microgram
mg	:	Milligram
µl	:	Microliter
µM	:	Micromolar
mM	:	Millimolar
mL	:	Milliliter
µm	:	Micrometer
pmol	:	pico moles
aac	:	Aminoglycoside acetyl transferase gene
AMR	:	Antimicrobial resistance
ARGs	:	Antimicrobial resistance genes
ATCC	:	American Type Culture Collection
bp	:	Base pairs
BHI	:	Brain heart infusion
BPW	:	Buffered peptone water
CAMHB	:	Cation adjusted Muller Hinton broth
CC	:	Culture control
COL	:	Colistin
COT	:	Co-trimoxazole
CIP	:	Ciprofloxacin

CSE	:	Centre for Science and Environment
CTX	:	Cefotaxime
CLSI	:	Clinical Laboratory Standard Institute
DNA	:	Deoxyribonucleic acid
DHPS	:	Dihydropteroate synthetase
E. coli	:	*Escherichia coli*
EMB	:	Eosin methylene blue
ESBL	:	Extended spectrum beta lactamase
et al.	:	*et alia*
FRG	:	Fluoroquinolone resistant gene
IAEC	:	Institutional animal ethical committee
MDR	:	Multidrug resistance
min	:	minutes
NTC	:	Non template control
NFW	:	Nuclease free water
OD	:	Optical density
PBS	:	Phosphate Buffer Saline
PCR	:	Polymerase Chain Reaction
SRG	:	Sulfonamide resistant gene
TET	:	Tetracycline

Introduction

I. INTRODUCTION

The ever increasing demand for animal source of proteins globally has transformed the livestock production system from low scale extensive production system to more intensive system with a focus on use of locally available feed ingredients and antimicrobial growth promoters to maintain productivity and profitability resulting in an increase in Antimicrobial resistance (AMR). India has emerged as one of the major global hotspot for antimicrobial resistance, with increasing resistance rates to most antimicrobials in common pathogens. Recent studies in many parts of India have indicated high levels resistance in important pathogens against several classes of antibiotics in hospital and community settings. One of the major concerns in India apart from the AMR in human population has been the rampant use of antimicrobials as growth promoters in the food animal production system with no stringent implementation protocols (Van Boeckel *et al.*, 2015). In addition, livestock rearing system in India is generally family managed, wherein human and animals live in close proximity which further complicates the understanding of the pathways of AMR dissemination.

Small ruminants especially sheep and goat in India have played a major role in providing income and dietary protein to poor, small, marginal farmers and their families (Lalljee *et al*., 2019). The system of production which is mainly low input nomadic and semi-intensive system where in use of antibiotics is not well documented. Generally, farmers depend on para-vets or non-professionals rather than veterinarians for treatment due to accessibility and charges to be paid for professionals (Kumar and Gupta, 2018; Mutua *et al*., 2020). Hence, documentation of type, amount and duration antimicrobial usage and antimicrobial resistance in small ruminant production system is lacking.

In India, cross sectional studies on the prevalence of antimicrobial resistance has been well documented in poultry owing to its highly intensive production system with evidence of antimicrobial use (Brower *et al.*, 2017) and in pigs (Vinod Kumar *et al*., 2019). A study conducted by Parkar *et al.* (2014) observed that flies in poultry farm carried *Campylobacter*, whereas Wadaskar *et al*. (2019) detected *E. coli* and Salmonella in house flies trapped from the cattle, sheep, goat, poultry farms and veterinary clinical complex in Maharashtra. In similar lines, Bhushan *et al.* (2017) in their Centre for Science and Environment (CSE) report on antibiotic residues in poultry environment indicated prevalence of high level of multidrug resistance in *E. coli*, *K. pneumoniae* and *S. lentus* isolated poultry farm environment samples (poultry litter, poultry farm soil and nearby agricultural soil). They also opined that multidrug resistance is moving from farms to agricultural fields as evidenced by similar pattern of resistance in *E. coli* from both litter and agricultural soil. Therefore, the role of environment and vectors (flies, insects and rodents) cannot be underestimated in dissemination of AMR in farm ecosystem.

In recent past, to investigate antimicrobial resistance in healthy animals, commensal bacteria, such as *Escherichia coli*, are being globally used as an indicator to estimate AMR in livestock (Catry *et al*., 2016). In addition, in livestock species, the age of the animal has been reported as an important determinant of AMR carriage, wherein young animals have been found to carry higher levels of AMR in fecal *E. coli* isolates as compared to older animals (Springer *et al.*, 2019). Furthermore, faeces from the animals are an important source for spread of resistant organisms in the environment and eventually to humans, as manure from the farms is generally used to fertilize crops

produced inside the farms (Heuer *et al*., 2011). The composition of the population of *E. coli* isolates found in faeces may be affected by the geographic region or purpose of rearing, system of rearing (intensive/ semi-intensive/ extensive) and the antimicrobial treatments the animal has received (Duse *et al*., 2016). Depending on the life stage, animals are exposed to distinct nourishment, housing and antibiotic treatment, resulting in development of the antibiotic resistance and pool of resistance genes. However, literature search reveals paucity of information of AMR surveillance in entire livestock production chain taking to consideration the human-animal-environment interface to have better understanding of the complex pathways of AMR dissemination. Hence, longitudinal studies in entire livestock production system needs focus as it could provide better insights on the dynamics and shedding pattern of antimicrobial resistant commensal and/or pathogens.

Therefore, a comprehensive longitudinal study to determine the antimicrobial resistance in *E. coli* as indicator organism in intensive goat production chain as a model has been undertaken to answer few key questions *viz.*, whether antimicrobial resistance occurs in young animals at birth or do they acquire after birth, if so what could be the possible route or source (animal, environment samples or vectors), in order to provide scientific evidences on possible routes of dissemination of AMR in intensive goat production system with the following objectives:

1. To understand the temporal shedding pattern of antimicrobial resistant *E. coli* in young kids and their dams over a period of 6 months
2. Phenotypic and molecular characterization of antimicrobial resistance in *E. coli.*

Review of Literature

II. REVIEW OF LITERATURE

2.1 Antimicrobial use in food production system

The use of antimicrobial has become an integral part of modern intensive animal production systems especially for preventing and treating microbial pathogens, especially bacteria that cause animal diseases. Antibiotics are used in food animal production systems across the world for different purposes *viz.*, treatment, prophylaxis or prevention, metaphylaxis and growth promotion, by altering the microbial ecology of the intestinal tract (Looft *et al.*, 2012).

It has been estimated that of the various countries, the largest share of global antimicrobial consumption in food animal production system was reported in China (23%) followed by United States (13%), Brazil (9%), India (3%), and Germany (3%) and these five countries accounted for more than half of the global use of antimicrobials in animal production (Van Boeckel *et al.*, 2015).

Sahoo (2008) studied the antimicrobial usage pattern of Veterinarians in Odisha, India using a semi-structured questionnaire and observed that the most commonly prescribed drugs in dairy and poultry farming were β-lactam, fluoroquinolones and tetracyclines.

Newman *et al*. (2011) reported that the most commonly used antibiotic in animal husbandry sector in Ghana was tetracycline and penicillin. They attributed it to the easy

availability of these drugs in the market for a relatively long period of time and therefore have been extensively used among animals and humans.

Donkor *et al*. (2012) studied that antibiotic usage pattern in animal husbandry sector in Ghana and observed that 98 per cent of the livestock keepers used antibiotics on their animals. The major antibiotics used were penicillin, oxy-tetracycline, streptomycin, sulphonamides and. In addition they recorded that majority of the livestock keepers used antibiotics solely for treatment of animal diseases and none of them hardly used antibiotics for growth stimulation or promotion.

Eagar *et al.* (2012) conducted a survey in South Africa to determine antibiotic usage in food animals and reported that tetracyclines and beta-lactams were amongst the first four leading antibiotics commonly used.

Adesokan *et al*. (2015) studied the usage pattern of antimicrobial in livestock animals in south-western Nigeria and reported that tetracyclines (33.6%) were the most frequently used antimicrobial, followed by fluoroquinolones (26.5%), beta-lactams/ aminoglycosides (20.4%), macrolides (15.1%), furatadone (2.3%) and chloramphenicol (2.1%).

Bhowmik *et al*. (2017) studied the antibiotic prescription pattern of veterinarians for treatment in goats in Bangladesh and reported that the most commonly used antibiotic was streptomycin-penicillin (31.10%) followed by sulfadimidine (13.95%), Amoxicillin (11.39%) and a combination of gentamicin-sulfadiazine-trimethoprim (9.25%). They observed that Tylosin was the least prescribed antibiotic to goats (0.48%)

Kumar and Gupta (2018) reported that Dairy farmers in Haryana, India used antibiotics mainly for therapeutic purpose (83.92%) followed by sub-therapeutic use (8.93%) and prophylactic purpose (7.15%). They also opined that the decision making on selection of a particular antibiotic was influenced by their previous experience with the drug while managing similar symptoms its availability and advice by the veterinarians.

Subramanya *et al*. (2021) in their study on AMR pathogens in livestock and its environment in rural areas of Nepal, reported that the most commonly used antibiotic in animal sectors were tetracyclines, sulfa drugs, macrolides, polymyxins, bacitracin, nitrofurans, quinolones and aminoglycosides, whereas chloramphenicol was the least used antibiotic in the veterinary sector.

2.2 *Escherichia coli*

Escherichia coli are ubiquitous commensal in gastrointestinal tract of both food-producing animals and humans. Most strains of this enterobacterial species are harmless commensals that live in a mutually beneficial association with their hosts and seldom cause disease. *E. coli* because of its genetic flexibility and adaptability to constantly changing environments may play an important ecological role and can be used as a bioindicator of antimicrobial resistance (Stacy *et al*., 2014).

E. coli is intrinsically susceptible to almost all clinically relevant antimicrobial agents, but this bacterial species has a great capacity to accumulate resistance genes, mostly through horizontal gene transfer. The most problematic mechanisms in *E. coli* correspond to the acquisition of genes coding for extended-spectrum β-lactamases

(conferring resistance to broad-spectrum cephalosporins), carbapenemases (conferring resistance to carbapenems), 16S rRNA methylases (conferring pan-resistance to aminoglycosides), plasmid-mediated quinolone resistance (PMQR) genes (conferring resistance to fluoroquinolones), and *mcr* genes (conferring resistance to polymyxins) (Poirel *et al*., 2018).

2.3 Animal production system and its environment

2.3.1 Occurrence of *E. coli*

Lejeune *et al*. (2001) studied the role of cattle water trough as reservoir for *E. coli* and reported that an association existed between the presence of *E. coli* O157 in cattle water troughs and the infection status of cattle drinking from these troughs. They also opined that cattle frequently contaminated the water troughs with faeces or saliva containing and that contaminated troughs acted as long-term reservoirs of the organism with a real potential for infection of cattle weeks or months later.

Dargatz *et al*. (2005) conducted a study to evaluate the presence of *E. coli* in 1,000 ingredients and feed samples used for manufacture of cattle feed and observed that more than 48 per cent of the samples were contaminated. Similarly, da Costa *et al.* (2007) was able to recover *E. coli* from 32 and 50 per cent of the feed ingredients and mixed feed used for manufacture of poultry feed.

Mukherjee *et al*. (2004) observed that organic produce cultivated using cattle manure as fertilizer showed 16 per cent *E. coli* prevalence as compared to 7 per cent in

those which were cultivated using other type of animal manure-based fertilizer. They observed that use of cattle manure for fertilization in semi-organic and organic farms increased the risk of *E. coli* contamination by 2-fold and 7-fold, respectively.

Nkogwe *et al*. (2011) detected the frequency of occurrence of *E. coli*, *Salmonella* spp., and *Campylobacter* spp. in the faeces of wild rats (*Rattus* spp.) in Trinidad and Tobago and observed of a total of 204 trapped rats, intestinal contents were positive for *E. coli* (83.8%), *Campylobacter* spp. (3.4%), and *Salmonella* spp. (2.0%).

Marchant and Moreno (2013) conducted a study was to determine the dynamics and diversity of *Escherichia coli* populations in animal and environmental lines of a commercial farrow-to-finish pig farm in Spain along a full production cycle and observed that *E. coli* was prevalent in all the collected samples (pregnant sows, 1-week-old piglets, unweaned piglets, growers, and the finishers' floor pen and farm slurry tank). They also opined that the *E. coli* populations in the pig fecal microbiota and in the farm environment were highly dynamic and revealed a high level of diversity.

Himsworth *et al*. (2015) recorded the prevalence and characteristics of *Escherichia coli* in the faeces of wild urban Norway and black rats from Canada and observed that *E. coli* was detected in 397 of 633 (62.7%) urban rats.

Ercumen *et al.* (2017) evaluated the occurrence of *E. coli* in water, hands, food, flies and soil in Bangladesh and detected *E. coli* in source water (25%), stored water (77%), child hands (43%), food (58%), flies (50%), ponds (97%), and soil (95%). They opined that high prevalence of animal faeces and their associations with fecal

contamination in the domestic environment indicated that animals can be a major source of fecal pathogen exposure.

Sobur *et al*. (2019) carried out a study to determine load of *Escherichia coli* in dairy farm and its environmental components (cow dung – 15, milk – 10, milkers hand wash – 10, soil – 10 water – 5, and vegetables – 10) and observed that 75 per cent of the samples were positive for occurrence of *E. coli* and that the highest occurrence was recorded in soil samples (92.5 %) followed by vegetable (77.5%), milkers hand (72.5%), cattle faeces (78.33%), milk (67.5%) and lowest in water samples (45%).

Osei *et al.* (2019) identified various levels of microbial contaminants in water used in poultry farms in Ghana and reported that 91 per cent of the water used in poultry farms had various levels of microbial contamination with one or more microorganisms including *E. coli, S. typhi, S. aureus* and coagulase-negative *Staphylococci*.

Ong *et al*. (2020) studied the occurrence of *Escherichia coli* from Wild Birds and Rodents in Singapore and observed that *E. coli* was detected in 27.1 and 14.8 per cent in wild birds and rodents, respectively.

Manishimwe *et al*. (2021) in their study observed that the isolation rate of *E. coli* from dairy cattle faeces in Texas was 100 per cent based on growth in EMB and biochemical characterization.

2.3.2 AMR *E. coli*

Livestock operations that use antibiotics are closely associated with increases in antibiotic-resistant bacteria in animal caretakers, meat processors, and others who live in the vicinity of livestock facilities (Kozak *et al.*, 2009).

Khachatryan *et al.* (2004) observed that resistant strains out competed susceptible strains in calves but not in older cattle. However, the resistance genes were not by themselves associated with the higher fitness of AMR *E. coli* in the calves' GI tract

Duse *et al.* (2016) studied the occurrence and Spread of Quinolone-Resistant *Escherichia coli* (QREC) on Dairy Farms and its environment and reported that QREC was isolated from 60 per cent (calf) and 28 percent (cow) of the fecal samples and 44 per cent of the environmental samples. They opined that QREC circulates between cattle and the farm environment and that calves are important for the maintenance of QREC.

Machado *et al*. (2008) estimated the prevalence of extended-spectrum β-lactamases among *Enterobacteriaceae* isolates recovered from chickens and swine in Portugal and obsereved that 5.7 per cent of fecal samples from 35 healthy pigs and 10 per cent of those from healthy chickens were positive for ESBL- *E. coli.*

Ji *et al*. (2012) evaluated the presence of antibiotic residues and resistance in soil of the farmland near the hog, cattle and chicken farms in China and reported that the content of chloramphenicol, tetracycline and sulfonamide antibiotics was high.

Watson *et al*. (2012) conducted a longitudinal analysis of the epidemiology of ESBL-resistance in *E. coli* on a dairy farm in the UK. In that study, ESBL producing *E. coli* was found in calf faecal samples and persistently in water troughs in the calving pen, but only occasionally in those in the dry cow pen. Moreover, such strains were persistently recovered from pen walls in the calving- and calf pens. Cows were also more likely to shed ESBL-producing *E. coli* after compared to before calving. They suggested that transition via the calving area may be a crucial pathway for the dissemination of ESBL-producing *E. coli* between different cattle categories.

Udikovic-Kolic *et al*. (2014) observed that soil treated with cattle manure had been reported to harbor higher abundance of β-lactam resistant bacteria as compared to untreated soil, revealing that manure-fertilized soil could harbor more antimicrobial resistant microbes.

Liu *et al*. (2018) observed that 96.1 per cent of the *E. coli* isolated from pigs in China was resistant to ampicillin, followed by amoxicillin-clavulanic acid (91.2%), sulfamethoxazole/trimethoprim (82%), oxytetracycline (74.3%), enrofloxacin (70%), gentamicin (61.4%), florfenicol (58.8%), ciprofloxacin (57.9%), and amikacin (52.2%).

Cao *et al*. (2019) studied the AMR pattern of *E. coli* isolated from fecal samples of dairy cattle in Pennsylvania and reported higher levels of resistance toward tetracycline (93%), sulfonamide (56%) and streptomycin (53%).

Subramanya *et al*. (2021) aimed to determine the gut colonization rate of ESBL-producing *Enterobacteriaceae* in gut of healthy humans, their reared animals, and the

environment in Nepal. They observed that 75.8, 79.5, 47.7, 51, 34.9 and 42 per cent of samples from adult humans, their children, cattle, 51 goats, poultry and environmental samples carried ESBL *Enterobacteriaceae*.

Manishimwe *et al*. (2021) evaluated the prevalence of antibiotic resistance among *Escherichia coli* isolated from dairy cattle faeces in Texas and observed that 43.5 and 25.9 per cent of the *E. coli* were resistant to cefotaxime and quinolone as detected by plating of samples in MacConkey agar supplemented with cefotaxime (1.0 μg/mL) and ciprofloxacin (0.5 μg/mL), respectively.

Poulin-Laprade *et al*. (2021) conducted a longitudinal study of pigs reared under various husbandry conditions and reported abundances of total *Enterobacteriaceae* and those resistant to CTX and TET were prevalent in feed, faeces and manure samples. The fecal samples from suckling piglets originating from all farm types had the most abundant *Enterobacteriaceae* and tetracycline-resistant (TET^R) colonies were frequently isolated from primary samples regardless of husbandry practice.

Gruel *et al.* (2021) conducted a cross sectional survey to understand antimicrobial use in pigs, beef cattle, and poultry on farms on Guadeloupe, French West Indies and to acquire data on AMR in *Escherichia coli* in these food-producing animals. They reported that tetracycline was the most commonly used antimicrobial, but its use was not correlated to *E. coli* resistance. Extended-spectrum β-lactamase (ESBL) *E. coli* isolates were detected in 7.3, 14.7 and 35.3 per cent of pigs, beef cattle and poultry, respectively.

It has been suggested that young animals are colonized with AMR *E. coli* from faeces of their mothers at birth (Watson *et al*. 2012). However, it has been documented that farm environment may be a more important source for colonizing strains (Yamamoto *et al*., 2013).

2.4 Occurrence of genes encoding AMR gene in animal production system

Hernandez *et al*. (2011) indicated that at present, three types of plasmid-mediated quinolone resistance (PMQR) genes and their variations have been more frequently reported in various bacterial pathogens around the world. These are the quinolone resistance determinant (*qnr*) genes (*qnr*A, *qnr*B, *qnr*C, *qnR*D and *qnr*S), variant aminoglycoside acetyl transferase gene [*aac(6´)-Ib-cr*] and efflux pumps-encoding genes (*qep*A and *oqx*AB).

Hartmann *et al*. (2012) observed that 18.3 per cent of the soil samples collected from Burgundy region in France, where beef cattle farms was densely were contaminated with ESBL-producing bacteria carrying $bla_{CTX\text{-}M}$ gene.

Sulfonamide resistance is primarily mediated by the *sul*1, *sul*2 and *sul*3 genes encoding dihydropteroate synthetase (DHPS) with a low affinity for sulfonamides (Yun *et al*., 2012).

Jones *et al*. (2013) indicated that mice in the farm premises as a source of ciprofloxacin resistant *E. coli* in turkey fattening flocks in UK. In addition, they also opined that presence of cephalosporin resistance in fattening flocks to be associated with

certain external biosecurity factors such as nearby piggery units and staff working in the farm premises.

The prevalence of ESBL-producing bacteria in swine farms has been reported to range from approximately 10 to 45 per cent and *E. coli* was the major ESBL producer (Geser *et al*. 2011; Liu *et al*. 2018). The most prevalent ESBL gene type at swine farms was $bla_{CTX\text{-}M}$, while other β-lactamase genes such as bla_{TEM}, bla_{SHV}, bla_{OXA} and bla_{KPC} were also identified (Liu *et al*., 2018).

Apostolakos *et al*. (2019) recorded a high prevalence of ESBL and plasmid mediated AmpC-type cephalosporinase-producing *E. coli* in broiler parent flocks (98.3%), which decreased (29.6%) during the laying period. The prevalence again increased to 69.2 per cent at the start of the production cycle in the fattening broilers, then dropped to 54.2 per cent in the last sampling right before slaughter.

Kong *et al*. (2019) investigated the prevalence of drug-resistant genes in fattening sheep and observed the relative abundance of aminoglycoside resistance genes (*aac*A4, *str*B), beta -Lactamase resistance genes (*bla*TEM), tetracycline resistance genes (*tet*O, *tet*Q), MLSB resistance genes (*erm*A, *erm*C), sulfonamide resistance genes (*sul*II), quinolone resistance genes (*qnr*S) and integrase gene (*int*-I) were higher.

Oh *et al*. (2020) studied the distribution of transmissible ARGs associated with resistance to streptomycin, sulfonamides and tetracyclines in *E. coli*, isolates from the faeces of newborn calves and their maternal colostrums and observed that *tet*B gene was

the most prevalent resistance gene detected (75.9%), followed by *sul3* (63.0%), *str*B (25.9%), *str*A (24.1%), and *tet*A.

Shabana and Al-Enazi (2020), investigated the prevalence of plasmid-mediated resistance in *E. coli* strains isolated from healthy and diarrheic sheep and goats in Saudi Arabia and observed that *qnr*B and *qnr*A were the most frequently encountered PMQR genes followed by *qnr*S gene in sheep and goat. They highlighted the importance of sheep and goats as reservoirs for the dissemination of MDR *E. coli* and resistance gene horizontal transfer.

Subramanya *et al.* (2021) observed $bla_{CTX\text{-}M}$ gene (77.6, 43.9, and 48 per cent) was predominant among ESBL-producing *Enterobacteriaceae* from the gut of healthy humans, their reared animals, and the environment, respectively.

It has been documented by researchers that the majority of ESBL-producing bacteria was *E. coli* and that $bla_{CTX\text{-}M}$ was the most predominant ESBL gene detected followed by bla_{TEM} and bla_{SHV} in poultry farm (Saliu *et al*., 2017), dairy farm (Mir *et al.* 2018), cattle abattoir (Geser *et al*., 2011) and in beef cattle (Mir *et al.*, 2016).

2.5 Longitudinal studies of AMR *E. coli* in animal production

A longitudinal study conducted on two dairy farms to investigate the pattern of shedding of verotoxin-producing *Escherichia coli* (VTEC) in goats demonstrated that the proportion of samples containing VTEC was higher for replacement animals and adults (85.7% and 78.7%, respectively) than for goat kids (25.4%) (Orden *et al.*, 2008).

Edrington (2012) observed higher presence of AMR *E. coli* in younger animals as compared to older animals and hypothesized that that young animals have an underdeveloped gut in terms of bacterial diversity and that resistant *E. coli* is able to compete successfully due to a possible linkage between resistance genes and genes conferring selective advantage in neonatal intestines. However, as the age of the animal advances, the bacterial microbiota diversifies and increases in numbers thereby resulting in loss of competitive advantage to the resistant *E. coli*, which is being slowly eliminated from the gastrointestinal tract.

Hansen *et al*. (2013) conducted a longitudinal study to elucidate carriage proportions and fecal counts of ESBL-producing *Escherichia coli* in pigs during the production cycle. They observed a significant decrease in carriage during the production cycle, with on average 50 per cent carriage immediately after birth, 58 per cent just before weaning, 29 per cent during weaning, and 12 per cent during finishing. In addition, a reduction in numbers of CTX-M-positive pigs was accompanied by a significant reduction in mean fecal counts of CTX-resistant coliforms.

Marchant and Moreno (2013) studied the dynamics and diversity of *Escherichia coli* populations in animal and environmental lines of a commercial farrow-to-finish pig farm in Spain along a full production cycle and observed that the *E. coli* populations in the pig fecal microbiota and in the farm environment are highly dynamic and show high levels of diversity.

It has been documented that individual-animal variation, the age of the animal (and characteristics of the intestine sections play a major role in determining the r the

differences and dynamic changes of *E. coli* intestinal populations in animals (Dixit *et al*., 2004; Schierack *et al*., 2009).

Ndegwa *et al*. (2019) studied the longitudinal shedding patterns and characterization of AMR *E. coli* in pastured goats and opined that colonization by bacteria resistant to antibiotics that had never been used on the farm indicated the role of environment in acquisition of resistant bacteria in goats.

Ndegwa *et al.* (2020) characterized the temporal dynamics in diversity of *E. coli* in fecal samples from a cohort of goat kids and adult meat goats on pasture over a one-year period. They observed that the prevalence of virulence genes and STEC was significantly higher in goat kids less than six months (76% /66%) than adults (48% /28%). They opined that higher prevalence in young animals might be attributed to the effect of weaning, which is a stressful period in young animals and is often associated with diarrhea in most farm animals.

Moor *et al*. (2021) conducted a longitudinal study to estimate point prevalence of extended-spectrum cephalosporin-resistant *Escherichia coli* (ESC R-Ec) by sampling individual pigs, pig farmers and the environment. They reported that ESC-R-Ec prevalence significantly decreased from 6.2 to 3.9 and 1.8 per cent for the suckling, weaned and fattening pigs, respectively.

Materials and Methods

III. MATERIALS AND METHODS

The present study was carried out to evaluate the occurrence of *E. coli* and its antimicrobial resistance in a selected goat farm of Hyadalu village, Kasaba hobli, Nelamangala taluk, Bengaluru rural district, Karnataka. Isolation and characterization of *E. coli* was carried out at Department of Livestock Products Technology, Veterinary College, Bengaluru, Karnataka to study the temporal shedding pattern of antimicrobial resistant *E. coli* in host related, environmental, human and rodent samples over a period of six months with special reference to tetracyclines, fluoroquinolones, Sulfonamides, Extended spectrum β-lactamase (ESBL) and colistin resistance, followed by molecular characterization of these isolates for the presence of genes encoding antimicrobial resistance in goat meat production system.

3.1 MATERIALS

3.1.1 General considerations

Chemicals and reagents used for the study were procured from M/s. Hi-Media Ltd., and M/s. Sigma Ltd. Glass wares used in the present study were procured from M/s. Borosil while the plastic ware procured from M/s. Tarsons Ltd. Oligonucleotide primers used in the present study were custom synthesized from M/s. Sigma-Aldrich Corporation, India. DNA Extraction kit from Favorgen was used.

Culture media, buffer and biochemical reagents were prepared using Distilled water in accordance with manufacturers' guidelines. Glassware used for the experiment

were prepared following established protocols (detergent soaking for overnight followed by washing in tap water, rinsing in distilled water, air drying, plugging with cotton, wrapping and sterilized by dry heat in hot air oven for one hour at 160°C).

Plastic ware like micropipette tip, micro centrifuge tube, and PCR tubes were sterilized by autoclaving at 15 psi at 121°C for 15 minutes. Culture media, broth were sterilized before use by autoclaving at 15 psi at 121^{o}C for 15 minutes.

3.1.2 Preparation of media

All the media used for isolation and identification of bacterial cultures were prepared following the standard guidelines of manufacturers. Detailed composition of the media and reagents used in this study are appended at the end.

3.1.3 Scientific equipment's used

Some of the important equipment used in this study was Incubator, Biosafety cabinet, refrigerated centrifuge, digital electronic balance, biological deep freezer, thermal cycler, horizontal electrophoresis unit and gel documentation system with image lab software.

3.2 PREPARATION OF BUFFERS AND CULTURE MEDIUM

3.2.1 Media, reagents and chemicals

The media and reagents were either obtained from Hi-media, Mumbai, Bengaluru or prepared in the laboratory as per the standard procedures. Media and reagents were prepared as recommended by the manufacturer.

3.2.2 Preparation of Brain Heart Infusion (BHI) Broth

Purpose of non-selective enrichment is to hasten proliferation and regeneration of *E. coli* cells. Representative sample was transferred to 10 ml BHI Broth and incubated at 37±1°C for 18-24 hours.

3.2.3 Nutrient broth with 30 % glycerol

a. Nutrient broth 70 ml

b. Sterile glycerol 30 ml

Glycerol was sterilized in hot air oven at 160^{o} C for one hour and added into sterile nutrient broth, mixed well and made aliquots into sterile cryovials of 1.8 ml capacity and stored at 4^{o} C for further use.

3.2.4 Preparation of Tetracycline (TET) solution (8 mg mL^{-1})

Tetracycline	80 mg
Milli-Q water	10 ml

- Micro-centrifuge tube of 1.5 mL capacity was tared on a weighing balance
- 80mg Tetracycline weighed in this micro-centrifuge tube

- One mL autoclaved Milli-Q water was added to it and mixed well by pipetting several times until it was dissolved completely to obtain a clear solution.

- This solution was transferred into a 15 mL falcon tube with 10 mL of autoclaved Milli-Q water to prepare Tetracycline solution of 8 mg mL^{-1} concentration.

- This solution was then filtered through a 0.22 μm syringe filter under sterile conditions and divided into 10 aliquots of 1 mL volume each in 1.5 mL micro centrifuge tubes and stored at -20℃ for future use.

3.2.4.1 Preparation of EMB agar with TET (2μg mL^{-1}) plates

- To 1000 mL of sterile EMB at ~50-55 °C, 100μL of TET solution (8 mg mL^{-1}) was added under laminar air flow.

- Swirled well to mix without creating any air bubbles.

- The media was poured onto petri dishes and allowed to dry.

- The agar plates were packed into petri dish bags and stored at 4°C for future use.

3.2.5 Preparation of Sulfonamide (Co-trimoxazole-COT) solution (4 mg mL^{-1})

Co-trimoxazole	40 mg
Milli-Q water	10 ml

- Micro-centrifuge tube of 1.5 mL capacity was tared on a weighing balance

- 40mgCo-trimoxazole weighed in this micro-centrifuge tube

- One mL autoclaved Milli-Q water was added to it and mixed well by pipetting several times until it dissolved completely to obtain a clear solution.

- This solution was transferred into a 15 mL falcon tube with 10 mL of autoclaved Milli-Q water to prepare Sulphonamide (Co-trimoxazole) solution of 4 mg mL^{-1} concentration.

- This solution was then filtered through a 0.22 μm syringe filter under sterile conditions and divided into 10 aliquots of 1 mL volume each in 1.5 mL micro centrifuge tubes and stored at -20℃ for future use.

3.2.5.1 Preparation of EMB agar with COT (4μg mL^{-1}) plates

- To 1000 mL of sterile EMB at ~50-55 °C, 100μL of COT solution (4 mg mL^{-1}) was added under laminar air flow.

- Swirled well to mix without creating any air bubbles.

- The media was poured onto petri dishes and allowed to dry.

- The agar plates were packed into petri dish bags and stored at 4°C for future use.

3.2.6 Preparation of Ciprofloxacin (CIP) solution (0.5 mg mL^{-1})

Ciprofloxacin	5 mg
Milli-Q water	10 ml

- Micro-centrifuge tube of 1.5 mL capacity was tared on a weighing balance
- 5mg Ciprofloxacin weighed in this micro-centrifuge tube
- One mL autoclaved Milli-Q water was added to it and mixed well by pipetting several times until it dissolved completely to obtain a clear solution.
- This solution was transferred into a 15 mL falcon tube with 10 mL of autoclaved Milli-Q water to prepare Ciprofloxacin solution of 0.5 mg mL^{-1} concentration.
- This solution was then filtered through a 0.22 μm syringe filter under sterile conditions and divided into 10 aliquots of 1 mL volume each in 1.5 mL micro centrifuge tubes and stored at -20℃ for future use.

3.2.6.1 Preparation of EMB agar with CIP (0.5μg mL^{-1}) plates

- To 1000 mL of sterile EMB at ~50-55 °C, 100μL of CIP solution (0.5 mg mL^{-1}) was added under laminar air flow.
- Swirled well to mix without creating any air bubbles.

- The media was poured onto petri dishes and allowed to dry.

- The agar plates were packed into petri dish bags and stored at 4°C for future use.

3.2.7 Preparation of Colistin (COL) solution (2 mg mL^{-1})

Colistin	20 mg
Milli-Q water	10 ml

- Micro-centrifuge tube of 1.5 mL capacity was tared on a weighing balance

- 20mg Colistin weighed in this micro-centrifuge tube

- One mL autoclaved Milli-Q water was added to it and mixed well by pipetting several times until it dissolved completely to obtain a clear solution.

- This solution was transferred into a 15 mL falcon tube with 10 mL of autoclaved Milli-Q water to prepare Colistin solution of 2 mg mL^{-1} concentration.

- This solution was then filtered through a 0.22 μm syringe filter under sterile conditions and divided into 10 aliquots of 1 mL volume each in 1.5 mL micro centrifuge tubes and stored at -20℃ for future use.

3.2.7.1 Preparation of EMB agar with COL (2μg mL^{-1}) plates

- To 1000 mL of sterile EMB at ~50-55 °C, 100μL of COL solution (1 mg mL^{-1}) was added under laminar air flow.

- Swirled well to mix without creating any air bubbles.

- The media was poured onto petri dishes and allowed to dry.
- The agar plates were packed into petri dish bags and stored at 4°C for future use.

3.2.8 Preparation of Cefotaxime (CTX) solution (2 mg mL^{-1})

Cefotaxime sodium salt	20 mg
Milli-Q water	10 ml

- Micro-centrifuge tube of 1.5 mL capacity was tared on a weighing balance
- 20mg Cefotaxime sodium salt weighed in this micro-centrifuge tube
- One mL autoclaved Milli-Q water was added to it and mixed well by pipetting several times until it dissolved completely to obtain a clear solution.
- This solution was transferred into a 15 mL falcon tube with 10 mL of autoclaved Milli-Q water to prepare Cefotaxime solution of 2 mg mL^{-1} concentration.
- This solution was then filtered through a 0.22 µm syringe filter under sterile conditions and divided into 10 aliquots of 1 mL volume each in 1.5 mL micro centrifuge tubes and stored at -20℃ for future use.

3.2.8.1 Preparation of EMB agar with CTX (2µg mL^{-1}) plates

- To 1000 mL of sterile EMB at ~50-55 °C, 100µL of CTX solution (1 mg mL^{-1}) was added under laminar air flow.

- Swirled well to mix without creating any air bubbles.
- The media was poured onto petri dishes and allowed to dry.
- The agar plates were packed into petri dish bags and stored at 4°C for future use.

3.2.9 Quality control of the prepared media

- **pH:** The pH of each batch of the media prepared was checked using a small amount of the medium in the laminar hood and checked for its pH using a pH paper. Media was discarded when the pH requirements were not met.
- **Sterility checking -** After pouring the plates, the plates were kept at 37^{o} for 12-16 Hrs. The plates were discarded if it had any colonies after incubation.

3.3 METHODS

3.3.1 Study area and period

A commercial stall-fed goat farm located in Hyadalu village, Kasaba hobli, Nelamangala taluk, Bengaluru rural district, Karnataka was selected for the present study. The farm is located at latitude of 13.1356508° N and longitude of 77.4115271° E. The overall farm structure and dimension of the individual pen are presented in Plate 3.1 and 3.2). The present study was carried out from December 2020 to July 2021. The study was carried out with permission from Institutional Animal Ethics Committee (VCH/IAEC/2020/14)

Plate 3.1: GPS coordinates and overall layout of the goat farm of the present study

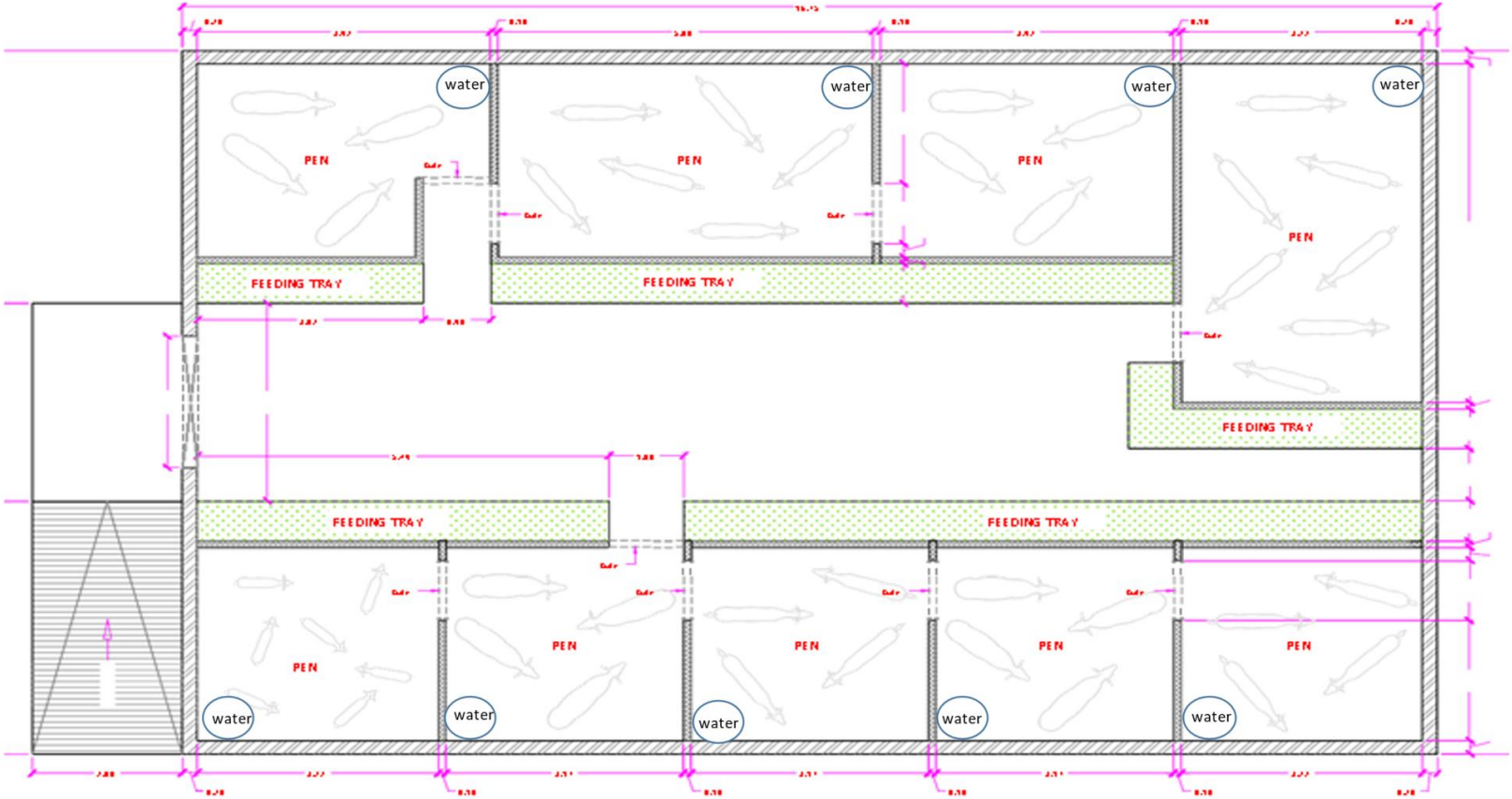


Plate 3.2: Inside view and shed dimensions

3.3.2 Data collection

Preliminary data regarding various farming practices, antimicrobial usage and managemental practices of the goat farm was collected using a structured questionnaire (Annexure 1). The antibiotic treatment of the animals recruited for the present study was recorded regularly during the entire study period.

3.3.3 Animals used and Samples collected

In the present study, six full term pregnant does were selected and 12 kids from these does were used. Immediately after kidding, kids and does were identified by applying tags. A total of 690 samples (Rectal swabs from does and kids, milk sample from does till weaning, fodder, feed concentrate, water samples from tank and trough, boot socks sample, human handler swab, human toilet seat swab and rat fecal sample) were collected from the day of kidding (day 0) at weekly intervals for a period of 24 weeks (Fig. 3.1). The samples consisted ofrectal swabs from Kids (n=288) and does (n=144), milk sample (n=66), fodder (n=24), Concentrate feed (n=24), tank water (n=24), trough water(n=24), handler hand swab (n=24), human toilet sample (n=24), boot socks (n=24) and rodent faecal sample (n=24).The collected samples were immediately transported to the Department of Livestock Products Technology, Veterinary College, Bengaluru under cold chain for isolation and identification of the organism.

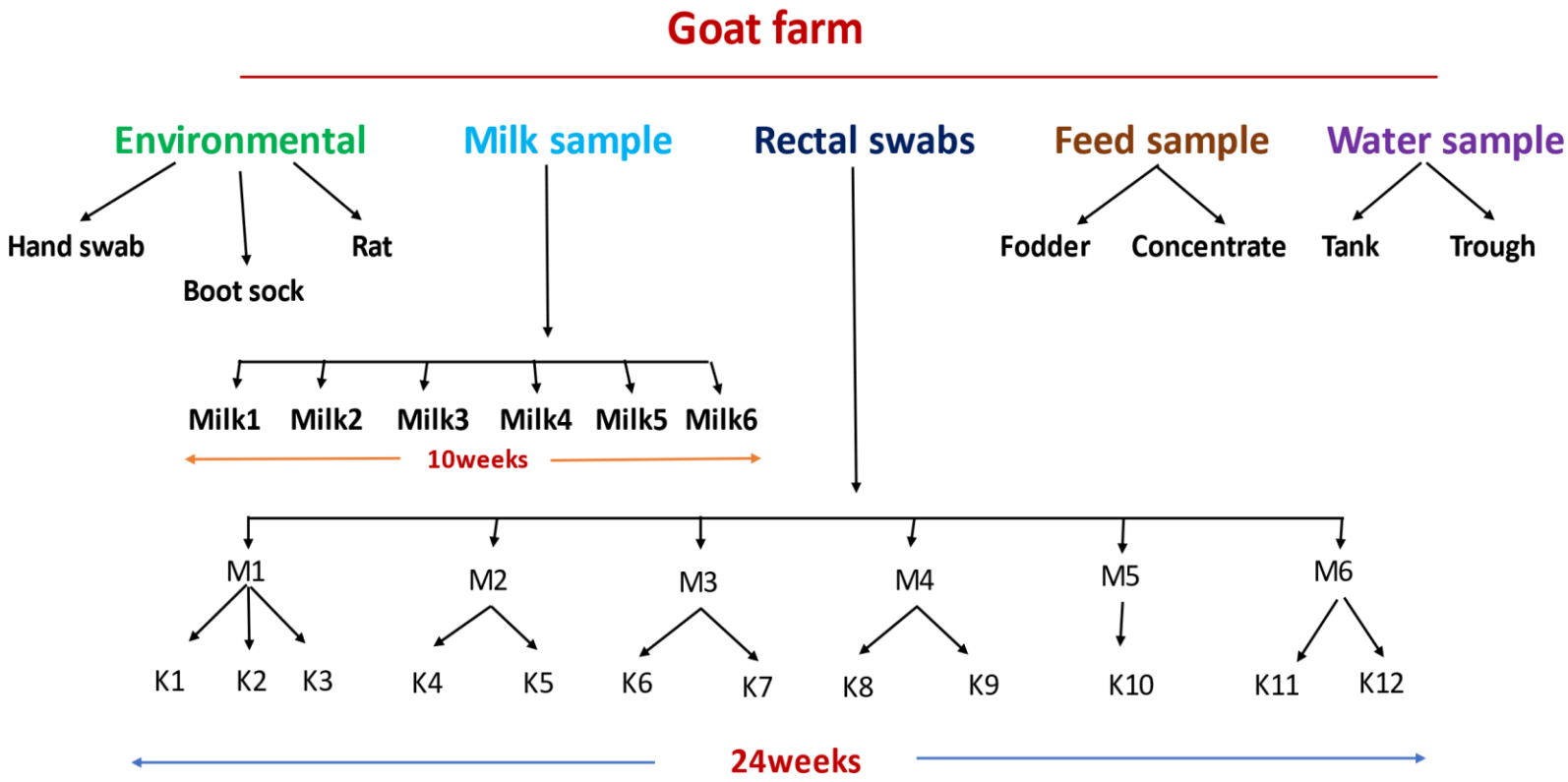


Fig. 3.1: Sample used for isolation of *E. coli* in goat meat production system

3.3.3.1 Rectal swabs

1. Initially, the swabs were immersed in sterile BPW before sampling. Rectal Swabs from kids and does were swabbed using sterile swab/animal in the goat farm. Immediately after sampling, the swabs were transferred to 10 ml container containing sterile BHI broth for enrichment and were transported to laboratory in ice box containing ice packs.

2. In the laboratory, swabs immersed in the BHI broth container and were incubated for 37°C for 18-24 Hrs (hrs).

3. A total of 432 rectal swabs (288 from kids and144 from does) were collected during the study period.

3.3.3.2 Milk sample

Milk samples were collected directly from the does udder using sterile 15ml centrifuge tubes and were transported to laboratory in ice box. In the laboratory, 1ml of milk sample was transferred to 9ml of BHI broth and was incubated for 37°C for 18-24 hrs.

3.3.3.3 Water sample

Water samples were collected from two different point *viz*., water storage tank and from the water trough placed inside the shed. Approximately 10-15 ml of water

sample was collected in sterile containers and transported to laboratory in cold chain (Shecho *et al.,* 2017).

3.3.3.4 Fodder and Concentrate Sample

1. Approximately, 50gm of fodder samples were collected wearing sterile gloves which then transferred to a sterile zip lock cover and transported to the laboratory under cold chain. In the laboratory, the sample was transferred to BHI broth and was incubated for 37°C for 18-24 Hrs.

2. Approximately, 50 gm of concentrate sample was taken using sterile spatula and transferred to sterile zip lock cover. In the laboratory, the 5-10 gm of sample was transferred to BHI broth and incubated for 37°C for 18-24 Hrs.

3.3.3.5 Human toilet sample

Sterilized cotton swabs immersed in sterile BPW were used to sample an area of $10cm^2$ of the human toilet seat and immersed in sterile BHI broth was transported to laboratory in ice box and was incubated at 37 ˚C for 18-24 Hrs.

3.3.3.6 Rodent faecal sample

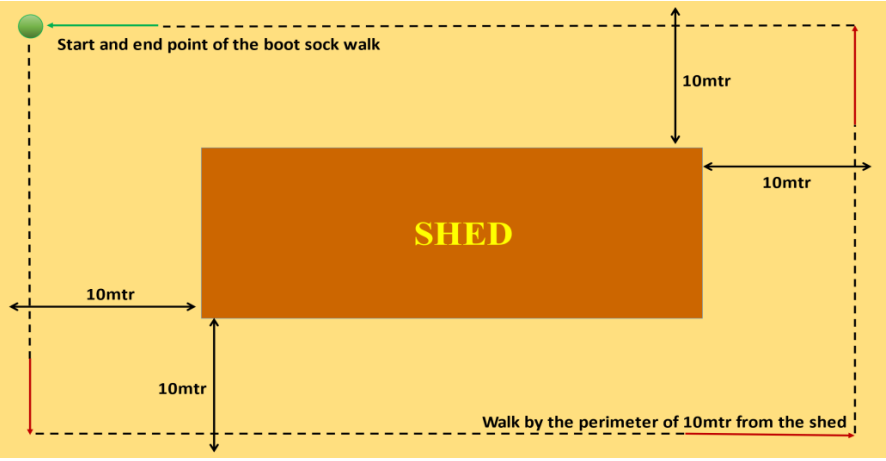
Rat faecal sample is collected by trapping the rats in Sherman rat trap and the faecal droppings inside the trap were collected by wearing sterile gloves which then transferred to a sterile zip lock cover and were transported to laboratory in ice box. The

faecal droppings were transferred to 10ml of BHI broth and were incubated at 37°C for 18-24 hours.

3.3.3.7 Farm external soil sampling

Before sampling, 10 ml of BPW was added to sterile boot socks in a sterile zip-lock bag. While collecting samples, the soaked boot socks was wore over the plastic shoe covers (Plastic boot covers over the shoes was to avoid any direct contact of the boot socks with the shoes) and walked around the perimeter of the farm in a straight-line at a distance of 10 m from the sheds.

After sampling the boot socks were collected and kept in the same zip lock bag and transported to lab. In the lab, outer surface of the zip lock bags was wiped with 70 % ethanol and then 50 mL of sterile BHI was added into these bags containing the boot socks and mixed well manually by palpating the bag. Approximately 10 ml was removed and incubated at 37 ˚C for 18-24 Hrs.



3.3.3.8 Hand swab from worker

Worker directly in contact with the animals were swabbed in an area of 2 cm^2 and the swabs immersed in sterile BHI broth and transported to laboratory in ice box and was incubated at 37 ˚C for 18-24 hrs. A written consent was obtained before collection of human hand swabs.

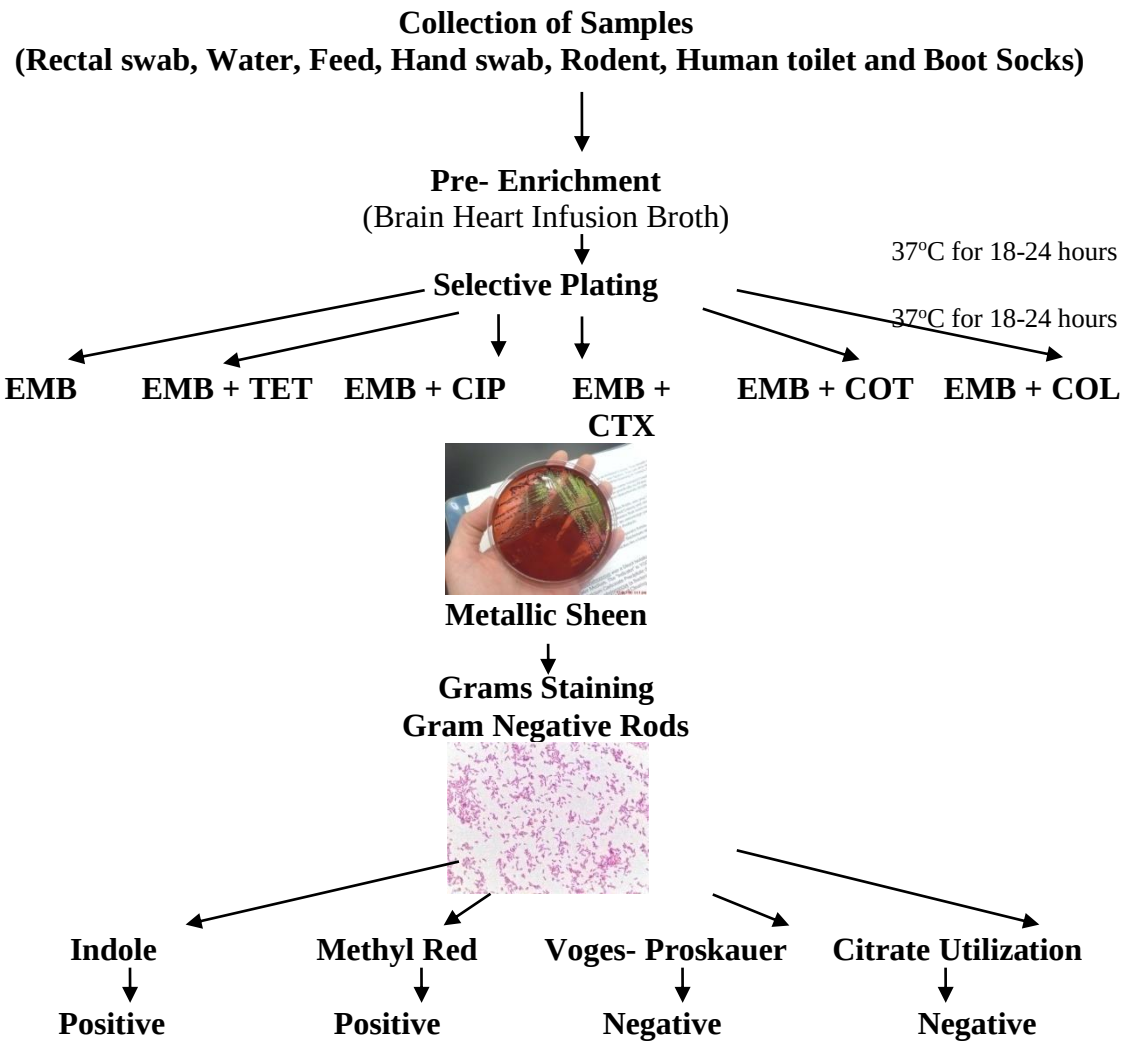


Plate 3.3: Flow chart of the study design

3.4 STUDY DESIGN:

To understand the temporal shedding pattern of antimicrobial resistant *E. coli* in young kids and their dams over a period of 6 months in goat meat production chain, the study design was categorized into different stages:

Stage I	:	Isolation and identification of *E. coli* by streaking the samples on to Eosin Methylene blue (EMB) agar with concentration of different antibiotics (Tetracycline, Sulfonamides, fluoroquinolones, ESBL and colistin) based on morphological, cultural and biochemical characteristics in accordance with the standard procedure.
Stage II	:	Molecular confirmation of the *E. coli* isolates based on species specific Polymerase Chain Reaction (PCR).
Stage III	:	Molecular characterization of Tetracycline, Sulphonamides, Fluoroquinolones, ESBL and colistin resistant *E. coli* for presence of genes encoding resistance based on PCR

3.4.1 Isolation, identification and confirmation of *E. coli* from samples

A. Non-selective pre-enrichment (liquid medium)

B. Selective plating (solid media)

C. Confirmation of *E. coli*

3.4.1.1 Non-selective pre-enrichment:

Purpose of non-selective enrichment is to hasten proliferation and regeneration of *E. coli* cells. Representative sample will be transferred to 10 ml BHI Broth and incubated at 37±1°C for 18-24 hours.

3.4.1.2 Selective plating:

Isolation of *E. coli* was done from the enriched samples in BHI obtained by incubation at 37°C. After enrichment one loopful culture was streaked onto EMBA, EMBA with Tetracycline (EMBA + TET), EMBA with Sulphonamide (EMBA + COT), EMBA with Ciprofloxacin (EMBA + CIP), EMBA with Cefotaxime (EMBA + CTX) and EMBA with Colistin (EMBA + COL).The plates were then incubated at 37±1 °C for 18-24 hrs and checked for colonies with production of metallic sheen typical to the genus *E. coli.* Typical metallic sheen colonies were streaked on to the nutrient agar slants and incubated at 37˚C for 24 h. Simultaneously culture with typical colony characteristics from each EMB plates were preserved in 30% glycerol solution at -18 ^{0}C. Slant culture emerging from a single isolated colony was further subjected to the biochemical conformation.

3.4.1.3 Biochemical Characterization of *E. coli*

3.4.1.3.1 Gram's staining

The test organisms were stained by Gram's Method to determine their staining characteristics and purity of the culture. All the isolates were observed for gram negativity, shape, size, conformation, arrangement patterns.

3.4.1.3.2 Indole Test:

Test tube containing 3 mL of tryptone water were inoculated with culture isolate and incubated at 37°C for 48 h. After incubation, 0.5 mL of Kovac's reagent was added and formation of red ring on top of the broth was considered as a positive for indole reaction and no change in color was taken as negative reaction.

3.4.1.3.3 Methyl red test:

Tube containing sterilized MR-VP medium was inoculated with the culture isolate and incubated at 37°C for 24 h. After incubation, two drops of methyl red indicator was added and development of red color was taken as a positive reaction, while no change in the color was taken as negative.

3.4.1.3.4 Voges-Proskauer test:

Tubes containing MR-VP medium were inoculated with culture isolate and incubated at 37^{o}C for 48 h. After incubation, 0.6 mL 5% α-napthol solution and 0.5 mL 40 % aqueous potassium hydroxide solution were added followed by thorough mixing. Positive reaction was indicated by formation of deep pink red colour within an hour of addition and no change in colour of the tubes were taken as negative.

3.4.1.3.5 Citrate utilization test:

Simmon's citrate agar was inoculated with the culture onto the slope and incubated at 37^{o}C and change in colour of the medium from green to blue coupled with growth along the streak line (Negative test) will be examined daily for up to seven days.

3.4.2 Minimum inhibitory concentration (MIC) by broth micro dilution method

Standard broth micro dilution technique was used for studying colistin resistance of *E. coli* and Salmonella. Antibiotic was procured from M/s. HiMedia (Colistin sulphate; PCT1142) and Cation-adjusted Mueller-Hinton broth (CAMHB). The test was performed in untreated polystyrene flat bottom 96 well plates.

3.4.2.1 Preparation of antibiotics stock solution

Colistin stock solution was prepared at concentrations of 1000µg/mL dissolved in double distilled water and aliquoted in small quantities in Eppendorf tubes and stored at -20°C for further use. The antibiotic solution was frizzed thawed only one time.

Colistin solution was performed as per the following formula:

$$\text{Volume (ml)} = \frac{\text{Weight (mg)} * \text{Potency(µg/mg)}}{\text{Concentration (µg/ml)}}$$

$$\text{Weight mg} = \frac{\text{Volum (ml)} * \text{Concentration (µg/ml)}}{\text{Potency(µg/ml)}}$$

3.4.2.2 Preparation of the inoculum

Colony Suspension method was used for inoculum preparation, Direct broth suspension of 4-5isolated colonies selected from an 18- to 24-hour nutrient agar were touched at the top of each colony with a loop and transferred into sterile glass tube containing 4-5 ml sterile CAMHB. The suspension was adjusted to achieve turbidity equivalent to a 0.5 McFarland standard, the inoculum was used within 15 minutes.

3.4.2.3 Quality control

For quality control the experiment was consisting of the followings:

Test: CAMHB + bacterial culture + different concentration of colistin in duplicate

Sterility control: Sterile MHB without any colistin

Highest antibiotic control (HAC): CAMHB + 128µg mL^{-1} of colistin (to prevent bacterial growth due to presence of the antibiotic)

Highest antibiotic control (HAC): CAMHB + 128µg mL^{-1} of colistin

Lowest antibiotic control (LAC): CAMHB + 0.25 mL^{-1} of colistin

Culture control: MHB + bacterial culture

Positive control: CAMHB + *mcr1*gene positive bacterial culture in duplicate

3.4.2.4 Procedure

100µL sterile CAMHB was added to all the wells except antibiotic highest antibiotic concentration well. And to the first well 200µL antibiotic solution containing 128µg/µl was added to the first well and serial two-fold dilution of the antibiotic solution was made from 128 to 0.25µg/µL and 100µl was discarded form the last wells. Reconstituted 100µl 0.5 McFarland culture was added to the wells containing 100 µL

antibiotic dilution to achieve a final OD_{600} of 0.2 in the test wells (in 200µL total volume per well) (Fig. 3.2).

3.4.2.5 Reading and interpretation of broth micro dilution result

The MIC result was interpreted as lowest concentration of antimicrobial agent that completely inhibits organism growth in the wells as detected by the unaided eye. The amount of growth in the wells containing the antimicrobial agent was compared with the amount of growth in the growth-control wells (no antimicrobial agent) used in each set of tests when determining the growth end points.

To convert the turbidity in the plate to numeric data optical density (OD) was obtain by reading the bacterial growth in each well using in ELISA reader (ELISA reader Synergy H1 microplate reader, Biotek) at 600nm. The MIC value was registered for each bacterial isolate by determining the minimum concentration of colistin which inhibits any bacterial growth.

The cut-off value was interpreted as per the CLSI breakpoint (>2µg/mL for colistin sulphate) and the OD value of 0.1 at 600 nm was considered as the cut-off value for conversion into numeric data to determine the resistance or sensitivity of the isolates

Fig. 3.2: Plate layout for MIC broth microdilution method

		1	2	3	4	5	6	7	8	9	10	11	12
		128µg/mL	64µg/mL	32µg/mL	16µg/mL	8µg/mL	4µg/mL	2µg/mL	1µg/mL	0.5µg/mL	0.25µg/mL	CC[1]	SC[2]
Sample No. 1	A											CC	HAC3
	B											CC	HAC
Sample No. 2	C											CC	LAC[4]
	D											CC	LAC
Sample No. 3	E											CC	SC
	F											CC	SC
ATCC 25922 *	G											CC	SC
	H											CC	SC

* ATTC 25922 was used as a quality control and in one batch only in one plate in duplicate.

1. CC = Culture control (Media + Culture only)
2. SC = Sterility control (200µL CAMHB, Sterile Media only)
3. HAC =Highest concentration of antibiotics only (128µg/mL)
4. LAC = Lowest antibiotic concentration only (0.25µ/mL)

3.5 Genotypic characterization of antimicrobial resistance in *E. coli*

3.5.1 DNA Extraction Protocol

DNA extraction was done as per the manufacturer's instructions.

1. Loopful of culture was taken in a 1.5 ml Eppendorf tube along with 200µl of PBS.

2. 400 µl of digestion solution was added to sample and vortexed.

3. 3 µl of proteinase K solution was added and kept for incubation at 55˚C for 5 min in a water bath.

4. 260 µl of 100 % ethanol was added to this mixture.

5. This mixture was transferred into an EZ-10 spin column and centrifuged at 10,000 rpm for 2 min.

6. The flow through accumulated in the collection tube was discarded and 500 µl of wash solution was added followed by centrifugation at 10,000 rpm for 2 min.

7. The above step was repeated once again.

8. Flow through was discarded once again and centrifuged at 10,000 rpm for 1 min to remove residual wash solution.

9. The spin column was placed into a clean 1.5 ml Eppendorf tube.

10. A 30-50 µl of elution buffer was put into the centre part of membrane in the column and incubated at RT for 2-3 min.

11. Centrifugation at 10,000 rpm for 2 min was done to elute DNA from the column and was stored at -20ºC.

3.5.2 Confirmation of *E. coli* using PCR

All the *E. coli* isolates were confirmed by multiplex PCR targeting four housekeeping genes in suspected *E. coli* colonies: *uid*A (ß-D-galactosidase), *lac*Z (ß-D-galactosidase), *cyd*A (cytochrome bd complex) and *pho*A (bacterial alkaline phosphatase). Only when an isolate was found to be positive for all the above four gene, it was confirmed as *E. coli* and was used for further characterization. The primers used and the PCR conditions are presented in Table 3.1.

The reaction was performed in mixture of 25µL total volume/tube without the addition of DNA template. *Escherichia coli* (ATCC® 25922™) was used as reference strain for quality control and NFW as non-template control (NTC).

Table 3.1: Primers and PCR conditions for species specific PCR

Target gene	Sequence (5' to 3')	Size (bp)	Reference
LacZ	F: ATGAAAGCTGGCTACAGGAAGGCC R: GGTTTATGCAGCAACGAGACGTCA	264	Modak *et al.* (2012)
CydA	F: CCGTATCATGGTGGCGTGTGG R: GCCGGCTGAGTAGTCGTGGAAG	393	
UidA	F: AAAACGGCAAGAAAAAGCAG R: ACGCGTGGTTACAGTCTTGCG	147	
PhoA	F: GGTAACGTTTCACCGCAGAGTTG R: CAGGGTTGGTACACTGTCATTACG	468	

Cycling conditions (35 cycles)				
Initial Denaturation	**Denaturation**	**Annealing**	**Extension**	**Final extension**
94˚C for 5 min	94˚C for 30 s	60˚C for 30 s	72˚C for 45 s	72˚C for 5 min

3.5.3 Determination of antibiotic resistant genes in *E. coli* isolates

3.5.3.1 Tetracycline resistant genes

A multiplex PCR was carried out to determine the occurrence of genes encoding tetracycline (*tetA* and *tetM*) as per the protocol outlined by Sianglum *et al*. (2009) and the PCR condition and primer used are presented in Table 3.2.

3.5.3.2 Sulfonamide resistant genes

A multiplex PCR was carried out to determine the occurrence of genes encoding sulfonamide resistance (*sul1* and *sul2*) as per the protocol outlined by Jaja *et al.* (2020) and the PCR condition and primer used are presented in Table 3.2.

3.5.3.3 Fluoroquinolone resistant genes

Quinolone resistant genes were screened in *E. coli* using multiplex PCR in two sets based on the amplicon sizes of the gene primers (Table 3.3). The reaction was prepared for 19 µL total volume per tube without the addition of DNA template. Each run was included with positive controls, negative control and NTC. For NTC NFW was used instead of the template. PCR mix for one reaction was Taq 2x Master Mix 9.5µL, forward and reverse primers 0.5µL each primer and for each gene (10pmol), and nuclease free water 5.5µL. One (1) µL DNA template was added to the 19µl reaction mixture for a total volume of 20µL.

3.5.3.4 β lactase resistance genes (ESBL)

Genotypic characterization was carried out by PCR targeting four different genes that encodes ESBL in *E. coli* namely $bla_{\text{CTX-M}}$, bla_{SHV}, bla_{TEM} and bla_{OXA} genes using oligonucleotide primers and PCR conditions as given in Table 3.4. Simplex PCR amplification was carried out for gene targeting $bla_{\text{CTX-M}}$ and multiplex PCR was carried out targeting bla_{SHV}, bla_{TEM} and bla_{OXA}. The reaction was optimized in 25 µl PCR reaction mixture comprising of 12.5µl of 2X PCR Master mix, 1.0 µL (10 pmol to 20 pmol depending on the gene targeted) of each forward and reverse primers, 3µL of DNA template and Nuclease free water was added to make a final volume of 25µl. The PCR products were stored at 4˚C, until further use and analyzed by agarose gel electrophoresis.

3.5.3.5 Colistin resistant genes

For screening colistin resistance, genes conferring plasmid mediated (*mcr-1, mcr-2, mcr-3, mcr-4, mcr-5)* were targeted (Lescat *et al.*, 2018). The oligonucleotide primers and the cycling conditions are given in Table 3.5. PCR amplification was optimized in 25 µl PCR reaction mixture. The assay was performed in Eppendorf thermal cycler under standardized cycling conditions. The PCR products were stored at 4˚C, until further use and analyzed by agarose gel electrophoresis.

3.6 Statistical analysis

Data was analyzed using GraphPad Prism 5 software and chi-square test was carried out to determine the level of significance in occurrence of *E. coli* and AMR *E. coli* between weeks and between the different samples collected from the goat production system.

Table 3.2: Primer sequence and PCR cycling conditions for tetracycline and sulfonamide resistant genes

Group of antibiotics	Gene	PCR primer sequence (5′–3′)	Amplicon size (bp)	PCR cycling condition	References
Tetracyclines	***tet*A**	F- GCTACATCCTGCTTGCCTTC R- GGCAGGCAGAGCAAGTAGAG	280	Initial denaturation: 95°C/5mins 30 cycles of Denaturation: 95°C/1 min Annealing: 51°C/1 min Extension: 72°C/1 min Final extension: 72°C/7mins	Sianglum *et al.* (2009)
	***tet*M**	F- ACAGAAAGCTTATTATATAAC R- TGGCGTGTCTATGATGTTCAC	171		
Sulfonamide (COT)	***sul1***	F: TTCGGCATTCTGAATCTCAC R: ATGATCTAACCCTCGGTCTC	822	Initial denaturation: 94°C/5 mins 35 cycles of Denaturation: 94°C/45 sec Annealing: 55°C/45 sec Extension: 72°C/1.5 min Final extension: 72°C/5 mins	Jaja *et al.* (2020)
	sul2	F: CGGCATCGTCAACATAACC R: GTGTGCGGATGAAGTCAG	722		

Table 3.3: Primer sequence and PCR cycling conditions for fluoroquinolone resistant genes

Gene	PCR primer sequence (5′–3′)	Amplicon size (bp)	PCR cycling condition	References
***qnr*A**	F: CAGCAAGAGGATTTCTCACG R: AATCCGGCAGCACTATTACTC	631	Initial denaturation: 95°C/15mins 30 cycles of Denaturation: 94°C/30 sec Annealing: 55°C/90 sec Extension: 72°C/90 sec Final extension: 72°C/3 mins	Ciesielczuk *et al*. (2013)
***qnr*B**	F: GGCTGTCAGTTCTATGATCG R: GAGCAACGATGCCTGGTAG	489		
***Oqx*AB**	F: CCGCACCGATAAATTAGTCC R: GGCGAGGTTTTGATAGTGGA	313		
***qep*A**	F: GCAGGTCCAGCAGCGGGTAG R: CTTCCTGCCCGAGTATCGTG	218		
***qnr*D**	F: CGAGATCAATTTACGGGGAATA R: AACAAGCTGAAGCGCCTG	582		
***qnr*S**	F: GCAAGTTCATTGAACAGGGT R: TCTAAACCGTCGAGTTCGGCG	427		
aac (6')-Ib-cr	F: TTGGAAGCGGGGACGGAC R: ACACGGCTGGACCATA	260		
***qnr*C**	F: GCAGAATTCAGGGGTGTGAT R: AACTGCTCCAAAAGCTGCTC	118		

Table 3.4: Primer sequence and PCR cycling conditions for genes encoding ESBL

Gene	PCR primer sequence (5′–3′)	Amplicon size (bp)	PCR cycling condition	References
bla*$_{CTX-M}$ *(Universal)	F: ATGTGCAGCACCAGTAAAGTGATGGC R: TGGGTAAAGTAAGTCACCAGAATCAGCGG	593	Initial denaturation: 95°C/ 12 mins 30 cycles of Denaturation: 95°C/30 sec Annealing: 55°C/30s Extension: 72°C/1min Final Extension: 72°C/7mins	Boyd *et al.* (2004)
***bla*$_{TEM}$**	F: CATTTCCGTGTCGCCCTTATTC R: CGTTCATCCATAGTTGCCTGAC	800	Initial denaturation: 95°C/12mins 30 cycles of Denaturation: 95°C/30 sec Annealing: 55°C/30 sec Extension: 72°C/1 min Final Extension: 72°C/5 mins	Dallenne *et al.* (2010)
***bla*$_{SHV}$**	F: AGCCGCTTGAGCAAATTAAAC R: ATCCCGCAGATAAATCACCAC	713		
***bla*$_{OXA}$**	F: GGCACCAGATTCAACTTTCAAG R: GACCCCAAGTTTCCTGTAAGTG	564		

Table 3.5: Primer sequence and PCR cycling conditions for genes encoding colistin resistance

Gene	PCR primer sequence (5′–3′)	Amplicon size (bp)	PCR cycling condition	Reference
mcr 1	F: ATGCCAGTTTCTTTCGCGTG R: TCGGCAAATTGCGCTTTTGGC	502	Initial denaturation: 94°C/4 mins 30 cycles of Denaturation: 94°C/5 sec Annealing: 59°C/20 sec Extension: 72°C/1 min Final Extension: 72°C/5 mins	Lescat *et al.* (2018)
mcr 2	F: GATGGCGGTCTATCCTGTAT R: AAGGCTGACACCCCATGTCAT	379		
mcr 3	F: ACCAGTAAATCTGGTGGCGT R: AGGACAACCTCGTCATAGCA	296		
mcr 4	F: TTGCAGACGCCCATGGAATA R: GCCGCATGAGCTAGTATCGT	207		
mcr 5	F: GGACGCGACTCCCTAACTTC R: ACAACCAGTACGAGAGCACG	608		

Results

IV. RESULTS

The present study was carried out to understand the temporal shedding pattern of antimicrobial resistant *E. coli* in intensive goat production system over a period of 6 months in a single intensive goat farm located in Nelamangala taluk, Bengaluru rural district, Karnataka. *E. coli* was isolated from host samples (Kids, does and milk), as well as from environmental (feed, water and boot socks), human (hand swabs and toilet swabs) and rodent (fecal samples). The isolated *E. coli* was evaluated for antimicrobial resistance based on phenotypic methods and was subjected to conventional PCR for detection of genes encoding antimicrobial resistance. The results of the present study are presented in this section.

4.1 Antimicrobial usage and farm managemental practices

The antimicrobial usage in the farm under study and the managemental practices adopted were evaluated during the entire study period based on information collected personally by using questionnaire. The type of antimicrobial used in all the 6 mothers and 12 kids during the 24 weeks of study are presented as heat map (Fig. 4.1) and the per cent type of antibiotic used is presented graphically in Fig. 4.2 and the condition for which the antibiotics have been administered is presented in Table 4.1.

Based on the results it was evident that the animals were administered antibiotics frequently and it was observed that during the period of study Enrofloxacin was the most commonly used antibiotic accounting for 68 and 72 per cent in kids and does in the form of both oral and parental routes, followed by tetracycline (both oral and parental) in 24

and 20 per cent in kids and does, respectively. Sulfonamide in parental route has been used in 5 and 4 per cent in kids and does followed by combination of enrofloxacin (oral) and tetracycline (injectable) in 3 and 2 per cent of the kids and does. In addition, a combination of enrofloxacin (oral) and sulfonamide (injectable) have been used only in does (2%). It was also observed that antibiotics and their combination have been used to treat animals suffering from diarrhoea, respiratory symptoms, fever and anorexia. The owner of the farm used antibiotics only after consultation with veterinarian. Antibiotics were not used in the farm for growth promotion.

It was learnt that no withdrawal period was followed in any of the does after antibiotic treatment during the study period and the kids were allowed to feed on the milk from its mother, which has received antibiotic treatment. In addition, the manure generated from the farm was utilized for on-farm fodder (maize) and tree fodder (Subabul) cultivation and the fodder produced in the farm premises was only used for feeding of the animals in the farms. Open defecation was observed in the fodder plots by the children of the farm workers.

Table 4.1: Conditions encountered in the farm and the antibiotic administered

Condition	**Antibiotics used**		**Duration**
	Name	**Dosage & Route**	
Diarrhoea	Dirolin L tab (Metronidazole - IP - 1000mg, Furazolidone - IP - 500mg, Loperamide Hydrochloride - IP - 7.5mg)	½ tab, PO.	3days
	Enrotas BH liquid (Enrofloxacin 100mg + Bromhexine hydrochloride 7.5mg)	1ml/10kg BW PO	5days
	Biotrim Injection (Sulphadiazine IP: 400 mg Trimethoprim IP: 80 mg)	1ml/20kg BW, IM	3days
Respiratory	Enrotas BH liquid (Enrofloxacin 100mg + Bromhexine hydrochloride 7.5mg)	1ml/10kg BW, PO	5days
	Fortivir Inj (Enrofloxacin 100 mg)	1ml/10kg BW, IM	3days
Pyrexia	Oxytetracycline dihydrate 50mg/ml Inj	1ml/5kg BW, IM	1day

Week	K1	K 2	K 3	M 1	K 4	K 5	M2	K6	K7	M 3	K 8	K 9	M 4	K10	M 5	K11	K12	M 6
Week 1																		
Week 2																		
Week 3																		
Week 4																		
Week 5																		
Week 6																		
Week 7																		
Week 8																		
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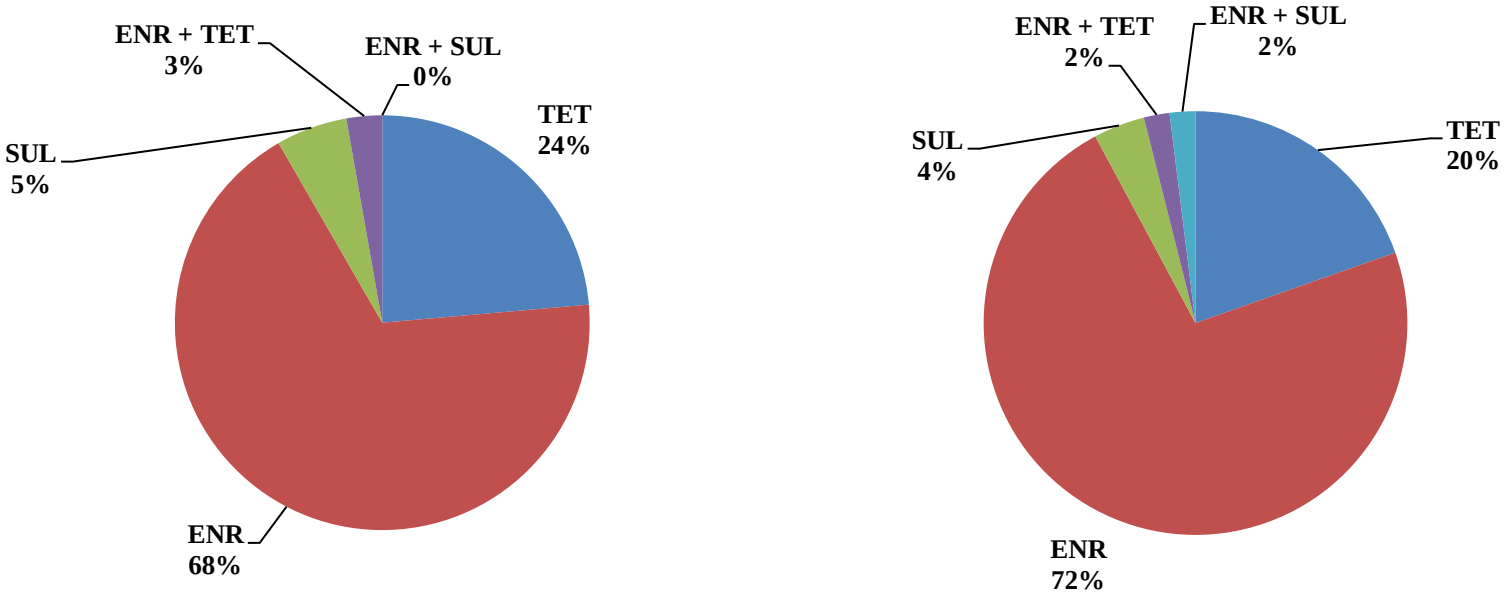
Tetracycline (TET) **Sulphonamide (SUL)** **Enrofloxacin (ENR)** **ENR + SUL** **TET+ ENR**

Fig. 4.1: Week wise and animal wise antibiotics used in goat production system



a) Kids **b) Does**

Fig. 4.2: Per cent of various antibiotics used in kids and does during the period of study

Note: ENR- Enrofloxacin; SUL- Sulfonamide or combination; TET- Tetracycline (oral/ parental)

4.2 Isolation and characterization of *E. coli*

All the 690 samples (Kids-288, Does- 144, Milk-66 and 24 each of fodder, concentrate, boot socks, water from tank and trough, hand swabs, toilet swabs and rodent fecal sample) were screened for presence of *E. coli* by streaking on to Eosin Methylene Blue agar (EMB) with different antibiotics. Isolates which developed characteristics metallic sheen were confirmed by using Gram's staining method and biochemical tests. The isolates which were gram negative rods and positive for catalase, Nitrate, Methyl Red and Indole tests and which were negative for oxidase, Voges Proskauer and citrate test were presumptively identified as *E. coli*. From each of the sample, one isolates each from EMB as well as from EMB with different antibiotic were used for further characterization. In total, 913 *E. coli* isolates (Kids-455, Does- 209, milk-34, Feed- 31, Water- 24, hand -17, human toilet- 50, boot socks- 48 and rodent- 45) identified by biochemical tests were subjected to PCR for amplification of four housekeeping genes specific for *E. coli*. The PCR and subsequent agarose gel electrophoresis revealed amplification of specific amplicon in all the isolates (Plate 4.1).

4.3 Sample wise occurrence of *E. coli* and AMR *E. coli* (Phenotype)

4.3.1 *E. coli*

The overall occurrence of *E. coli* from various samples from goat production system irrespective of the weeks of collection was 81.74 per cent (564/690). Among the various samples tested, 99.65 per cent (287/288) fecal sample of kids, 97.92 per cent (141/144) of fecal sample from does, 39.39 per cent (26/66) of milk samples, 58.33 per

cent (14/24) of fodder samples, 12.50 per cent (03/24) in feed concentrates, 25.00 per cent of water samples from over head tank, 54.00 per cent (13/24) of water sample from water trough, 46.00 per cent (11/24) from human hand swab, 92.00 per cent (22/24) of human toilet swabs, 88.00 per cent (21/24) of boot socks and 83.00 per cent (20/24) of rodent fecal sample (Table 4.2). A significant difference ($P \leq 0.05$) could be observed in the occurrence of *E. coli* from different samples with higher occurrence being recorded in kids, does, human toilet swabs, rodent samples and boot socks as compared to other samples.

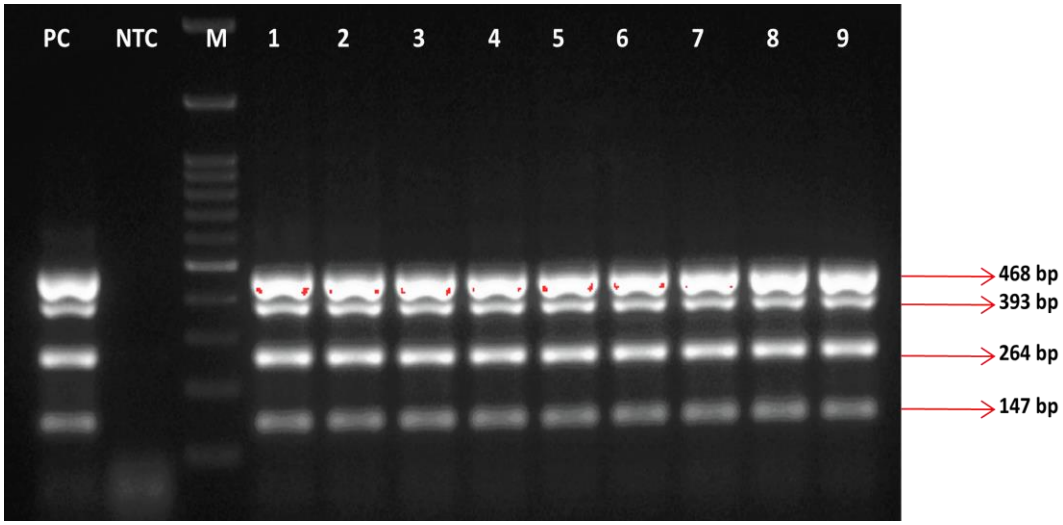


Plate 4.1: Amplification of four housekeeping gene specific for *E. coli*

PC: Positive control (*E. coli* ATCC 25922), NTC: Non-template control, M: 100 bp ladder; Lane 1-9: *E. coli* isolates from different samples from goat production system

4.3.2 Tetracycline resistant *E. coli*

The overall occurrence of tetracycline resistant *E. coli* in the present study was 42.75 per cent (295/690). Among the various samples tested 47.47 per cent (137/288) of fecal sample from kids, 40.28 per cent (58/144) of fecal sample from does, 18.18 per cent (12/66) of milk samples, 45.83 per cent (11/24) of fodder samples, 4.17 per cent (01/24) in concentrate, 16.70 per cent (04/24) of water samples from over head tank, 45.83 per cent (11/24) of water sample from water trough, 33.33 per cent (08/24) from human hand swab, 83.33 per cent (20/24) of human toilet swabs, 75.00 per cent (18/24) of boot socks and 62.50 per cent (15/24) of rodent fecal sample (Table 4.2). Occurrence of tetracycline resistant *E. coli* was highest in human toilet swab followed by rat fecal samples, kids, water trough and does ($P \leq 0.05$).

4.3.3 Fluoroquinolone resistant *E. coli*

The occurrence of Fluoroquinolone resistant *E. coli* was 31.25 per cent (90/288) in kids, 25.69 per cent (37/144) in does, 13.64 per cent (09/66) of milk samples, 45.83 per cent (11/24) of fodder samples, 4.17 per cent (01/24) in concentrate, 8.33 per cent (02/24) in water samples from over head tank, 20.83 per cent (05/24) in water sample from water trough, 25.00 per cent (06/24) from human hand swab, 66.67 per cent (16/24) of human toilet swabs, 66.67 per cent (18/24) of boot socks and 70.83 per cent (17/24) of rat fecal were found to identified as *E. coli* (Table 4.2). The overall occurrence of Fluoroquinolone resistant *E. coli* was 30.44 (210/690). It was observed that a significantly higher ($P \leq 0.01$) fluoroquinolone resistant *E. coli* was observed in fecal sample of rat followed by human

toilet, boot socks, whereas lowest occurrence was observed in concentrate and over head tank water samples.

4.3.4 Extended spectrum β lactamase (ESBL) *E. coli*

The overall occurrence of ESBL *E. coli* in various samples from goat production system was 16.23 per cent (112/690). It was observed that none of the samples from milk, concentrate feed and water samples (trough and over head tank) was positive for ESBL *E. coli*. A highly significant (P≤ 0.01) occurrence was observed in rodent samples (45.83%), followed by boot socks and human toilet swab (41.67% each). However lower occurrence was recorded in hand swabs (4.17%) followed by does (15.97%), fodder (16.67%) and in kids (18.40%).

4.3.5 Sulfonamide resistant *E. coli*

In the present study it was observed that 48.26 per cent (333/690) of the samples were positive for presence of sulfonamide resistant *E. coli.* A significant difference (P≤ 0.05) among the various samples tested was observed with highest occurrence in samples from human toilet swab (79.17%), followed by boot socks (70.83%) and rodent samples (62.50%). The occurrence was 59.03 per cent (85/141) in does, 52.43 per cent (151/288) in kids, 41.67 per cent (10/24) in fodder, 37.50 per cent (9/24) in water trough and 33.33 per cent (8/24) in human hand swabs. It was observed that none of the samples from concentrate feed was positive for presence of sulfonamide resistant *E. coli*.

Table 4.2: Overall occurrence of *E. coli* in different samples collected from goat farm over a period of 6 months

Sl. No.	Type of sample	No. of Samples collected	Positive sample				
			EMB	EMB+TET	EMB + CIP	EMB + CTX	EMB + COT
1	Kids	288	287 (99.65)	137 (47.57)	90 (31.25)	53 (18.40)	151 (52.43)
2	Does	144	141 (97.92)	58 (40.28)	37 (25.69)	23 (15.97)	85 (59.03)
3	Milk Samples	66	26 (39.39)	12 (18.18)	09 (13.64)	-	16 (24.24)
4.	Fodder	24	14 (58.33)	11 (45.83)	11 (45.83)	04 (16.67)	10 (41.67)
5.	Concentrate feed	24	03 (12.50)	01 (4.17)	01 (4.17)	-	-
6.	Water tank	24	06 (25.00)	04 (16.70)	02 (8.33)	-	3 (12.50)
7.	Water trough	24	13 (54.00)	11 (45.83)	05 (20.83)	-	9 (37.50)
6	Human Hands swabs	24	11 (46.00)	08 (33.33)	06 (25.00)	01 (4.17)	8 (33.33)
7	Human Toilet swabs	24	22 (92.00)	20 (83.33)	16 (66.67)	10 (41.67)	19 (79.17)
8	Boot Socks	24	21 (88.00)	18 (75.00)	16 (66.67)	10 (41.67)	17 (70.83)
9	Rodent faecal samples	24	20 (83.00)	15 (62.50)	17 (70.83)	11 (45.83)	15 (62.50)
		690	**564 (81.74)**	**295 (42.75)**	**210 (30.44)**	**112 (16.23)**	**333 (48.26)**

*EMB- Eosin Methylene Blue Agar, TET- Tetracycline (8mg/L); CIP- Ciprofloxacin (0.5mg/L); CTX- Cefotaxime (2mg/L), COT- Cotrimoxazole (4mg/L). Note: Value within parenthesis indicates per cent

4.4 Week wise occurrence of *E. coli* and AMR *E. coli*

4.4.1 Host related samples

The week wise occurrence of *E. coli* and AMR *E. coli* in host related samples (Kids, does and milk) are presented in Table 4.2 to 4.6 and is graphical presented in Figure 4.3.

4.4.1.1 *E. coli*

No significant difference could be observed in the occurrence of *E. coli* in both kids and does during all the weeks, whereas a significant difference ($P \leq 0.05$) was observed in the occurrence with respect to milk sample wherein a decrease was observed at week 9 as compared to other weeks (Table 4.3). The overall occurrence of *E. coli* in host related samples was 91.16 per cent (454/498).

4.4.1.2 Tetracycline resistant *E. coli*

A significant difference ($P \leq 0.05$) could be observed in the tetracycline resistant *E. coli* between the host related samples with lower resistance being observed in milk samples followed by kids and does (Table 4.4). However, higher resistance could be observed in host related samples till week 8 and thereafter a decrease in resistance could be observed with increase in the weeks in all the host related samples.

4.4.1.3 Fluoroquinolone resistant *E. coli*

A significant difference ($P \leq 0.05$) could be observed in the week wise occurrence of fluoroquinolone resistant *E. coli* between the host related samples with lower occurrence in milk compared to kids and does (Table 4.5). The samples from kids had higher resistance compared to that of the does and with increase in the period lower resistance could be observed.

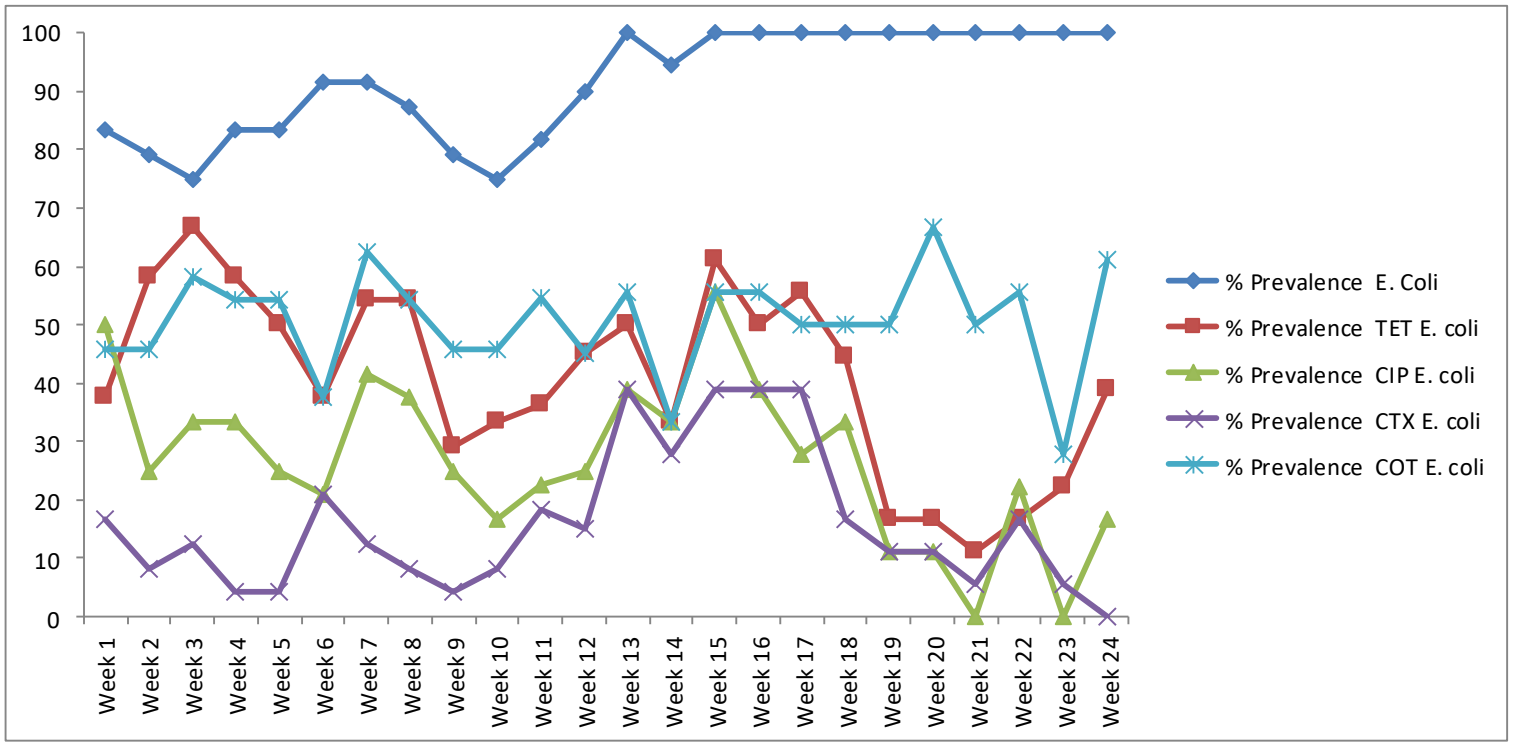


Fig. 4.3: Occurrence of *E. coli* and AMR *E. coli* in host related samples (Kids, does and milk) during the period of study (Week 1 to Week 24)

Table 4.3: Percent week wise occurrence of *E. coli* in host related samples (Rectal swabs from Kids, Does and Milk) collected from goat farm

Category	Kids (n=288)	Does (n=144)	Milk (n=66)	Week wise occurrence		
	%	%	%	Tested	Positive	%
Week 1	91.67	100	50	24	20	**83.33**
Week 2	100	83.33	53.3	24	19	**79.17**
Week 3	100	83.33	50	24	20	**75.00**
Week 4	100	100	66.7	24	22	**83.33**
Week 5	100	100	33.3	24	20	**83.33**
Week 6	100	100	66.7	24	22	**91.67**
Week 7	100	100	66.7	24	22	**91.67**
Week 8	100	100	50	24	21	**87.50**
Week 9	100	100	16.7	24	19	**79.17**
Week 10	100	100	0	24	18	**75.00**
Week 11	100	100	0	22	18	**81.82**
Week 12	100	100	0	20	18	**90.00**
Week 13	100	100	0	18	18	**100**
Week 14	100	83.33	0	18	17	**94.45**
Week 15	100	100	0	18	18	**100**
Week 16	100	100	0	18	18	**100**
Week 17	100	100	0	18	18	**100**
Week 18	100	100	0	18	18	**100**
Week 19	100	100	0	18	18	**100**
Week 20	100	100	0	18	18	**100**
Week 21	100	100	0	18	18	**100**
Week 22	100	100	0	18	18	**100**
Week 23	100	100	0	18	18	**100**
Week 24	100	100	0	18	18	**100**
	99.65 (287/288)	**97.92 (141/144)**	**39.39 (26/66)**	**498**	**454**	**91.16**

Table 4.4: Percent week wise occurrence of Tetracycline resistant *E. coli* in host related samples collected from goat farm

Week	Kids (n=288)	Does (n=144)	Milk (n=66)	Week wise occurrence		
	%	%	%	Tested	Positive	%
1	41.67	50.00	16.7	24	9	**37.50**
2	66.67	66.67	33.3	24	14	**58.33**
3	83.33	66.67	33.3	24	16	**66.67**
4	83.33	50.00	16.7	24	14	**58.33**
5	66.67	50.00	16.7	24	12	**50.00**
6	41.67	66.67	0	24	9	**37.50**
7	50.00	83.33	33.3	24	13	**54.17**
8	41.67	83.33	50	24	13	**54.17**
9	41.67	33.33	0	24	7	**29.17**
10	58.33	16.67	0	24	8	**33.33**
11	50.00	33.33	0	22	8	**36.36**
12	58.33	33.33	0	20	9	**45.00**
13	58.33	33.33	0	18	9	**50.00**
14	33.33	33.33	0	18	6	**33.33**
15	66.67	50.00	0	18	11	**61.11**
16	50.00	50.00	0	18	9	**50.00**
17	50.00	66.67	0	18	10	**55.56**
18	41.67	50.00	0	18	8	**44.44**
19	25.00	0	0	18	3	**16.67**
20	16.67	16.67	0	18	3	**16.67**
21	8.33	16.67	0	18	2	**11.11**
22	25.00	0	0	18	3	**16.67**
23	25.00	16.67	0	18	4	**22.22**
24	58.33	0	0	18	7	**38.89**
	47.57 (137/288)	**40.28 (58/144)**	**18.18 (22/66)**	**498**	**207**	**41.56 (207/498)**

Table 4.5: Per cent week wise occurrence of Fluoroquinolone resistant *E. coli* in host related samples (Rectal swabs from Kids, Does and Milk) collected from goat farm

Category	Kids (n=288)	Does (n=144)	Milk (n=66)	Week wise occurrence		
	%	%	%	Tested	Positive	%
Week 1	58.33	66.67	16.67	24	12	**50.00**
Week 2	25.00	33.33	16.67	24	6	**25.00**
Week 3	50.00	16.67	16.67	24	8	**33.33**
Week 4	50.00	16.67	16.67	24	8	**33.33**
Week 5	41.67	16.67	0	24	6	**25.00**
Week 6	33.33	0	16.67	24	5	**20.83**
Week 7	33.33	66.67	33.33	24	10	**41.67**
Week 8	41.67	50.00	16.67	24	9	**37.50**
Week 9	25.00	33.33	16.67	24	6	**25.00**
Week 10	25.00	16.67	0	24	4	**16.67**
Week 11	25.00	33.33	0	22	5	**22.73**
Week 12	33.33	16.67	0	20	5	**25.00**
Week 13	50.00	16.67	0	18	7	**38.89**
Week 14	33.33	33.33	0	18	6	**33.33**
Week 15	50.00	66.67	0	18	10	**55.56**
Week 16	41.67	33.33	0	18	7	**38.89**
Week 17	16.67	50.00	0	18	5	**27.78**
Week 18	33.33	33.33	0	18	6	**33.33**
Week 19	16.67	0	0	18	2	**11.11**
Week 20	16.67	0	0	18	2	**11.11**
Week 21	0	0	0	18	0	**0**
Week 22	25.00	16.67	0	18	4	**22.22**
Week 23	0	0	0	18	0	**0**
Week 24	25.00	0	0	18	3	**16.67**
	31.25 (90/288)	**25.69 (37/144)**	**13.64 (9/66)**	**498**	**136**	**27.31 (136/498)**

4.4.1.4 Extended spectrum β lactamase *E. coli*

The occurrence of ESBL *E. coli* week wise in host related samples reveled that none of the milk samples tested positive and that the occurrence rate was lower in both kids and does as compared to other antibiotic resistance studied. No significant difference could be observed in the occurrence of ESBL *E. coli*, during the different weeks, whereas a significant ($P \leq 0.05$) occurrence was observed between 13^{th} to 17^{th} weeks and during earlier and later weeks of the study lower occurrence could be observed (Table 4.6).

4.4.1.5 Sulfonamide resistant *E. coli*

No significant difference could be observed with respect to sulfonamide resistacne between kids and does, where as significantly lower occurrence was observed in milk samples till week 9 ($P \leq 0.05$). With respect to week wise occurrence of sulfonamide resistance similar high resistance pattern was observed throughout the period of study in both kids and does (Table 4.7).

Table 4.6: Per cent week wise occurrence of Cefotaxime resistant *E. coli* (ESBL) in host related samples (Rectal swabs from Kids, Does and Milk) collected from goat farm

Category	Kids (n=288)	Does (n=144)	Milk (n=66)	Week wise occurrence		
	%	%	%	Tested	Positive	%
Week 1	16.7	33.3	0	24	4	**16.67**
Week 2	8.33	16.7	0	24	2	**8.33**
Week 3	16.7	16.7	0	24	3	**12.50**
Week 4	8.33	0	0	24	1	**4.17**
Week 5	8.33	0	0	24	1	**4.17**
Week 6	33.3	16.7	0	24	5	**20.83**
Week 7	16.7	16.7	0	24	3	**12.50**
Week 8	8.33	16.7	0	24	2	**8.33**
Week 9	8.33	0	0	24	1	**4.17**
Week 10	8.33	16.7	0	24	2	**8.33**
Week 11	25	16.7	0	22	4	**18.18**
Week 12	16.7	16.7	0	20	3	**15.00**
Week 13	50	16.7	0	18	7	**38.89**
Week 14	25	33.3	0	18	5	**27.78**
Week 15	50	16.7	0	18	7	**38.89**
Week 16	41.7	33.3	0	18	7	**38.89**
Week 17	25	66.7	0	18	7	**38.89**
Week 18	16.7	16.7	0	18	3	**16.67**
Week 19	16.7	0	0	18	2	**11.11**
Week 20	16.7	0	0	18	2	**11.11**
Week 21	8.33	0	0	18	1	**5.56**
Week 22	8.33	33.3	0	18	3	**16.67**
Week 23	8.33	0	0	18	1	**5.56**
Week 24	0	0	0	18	0	**0**
	18.40 (53/288)	**15.97 (23/144)**	**0 (0/66)**	**498**	**70**	**14.05 (70/498)**

Table 4.7: Per cent week wise occurrence of Sulfonamide resistant *E. coli* in host related samples (Rectal swabs from Kids, Does and Milk) collected from goat farm.

Category	Kids (n=288)	Does (n=144)	Milk (n=66)	Week wise occurrence		
	%	%	%	Tested	Positive	%
Week 1	50.00	66.67	16.67	24	11	**45.83**
Week 2	50.00	66.67	16.67	24	11	**45.83**
Week 3	66.67	83.33	16.67	24	14	**58.33**
Week 4	66.67	66.67	16.67	24	13	**54.17**
Week 5	66.67	66.67	16.67	24	13	**54.17**
Week 6	41.67	50.00	16.67	24	9	**37.50**
Week 7	58.33	83.33	50.00	24	15	**62.50**
Week 8	50.00	66.67	50.00	24	13	**54.17**
Week 9	50.00	50.00	33.33	24	11	**45.83**
Week 10	58.33	50.00	16.67	24	11	**45.83**
Week 11	58.33	66.67	25.00	22	12	**54.54**
Week 12	50.00	50.00	0	20	9	**45.00**
Week 13	58.33	50.00	0	18	10	**55.56**
Week 14	33.33	33.33	0	18	6	**33.33**
Week 15	58.33	50.00	0	18	10	**55.56**
Week 16	50.00	66.67	0	18	10	**55.56**
Week 17	50.00	50.00	0	18	9	**50.00**
Week 18	41.67	66.67	0	18	9	**50.00**
Week 19	50.00	50.00	0	18	9	**50.00**
Week 20	58.33	83.33	0	18	12	**66.67**
Week 21	50.00	50.00	0	18	9	**50.00**
Week 22	58.33	50.00	0	18	10	**55.56**
Week 23	25.00	33.33	0	18	5	**27.78**
Week 24	58.33	66.67	0	18	11	**61.11**
	52.43 (151/288)	**59.03 (85/144)**	**24.24 (16/66)**	**498**	**252**	**50.60 (252/498)**

4.4.2 Week wise occurrence in Environment, human and rodent samples

The week wise occurrence of *E. coli* and AMR *E. coli* in environmental (feed, water, boot socks), human (hand and toilet swabs) and rodent samples are presented in Table 4.8 to 4.12 and is graphical presented in Figure 4.4.

A significant difference ($P \leq 0.05$) in occurrence of *E. coli*, tetracycline resistant *E. coli* and fluoroquinolone resistant *E. coli* could be observed between environmental, human and rodent samples, where in human toilet swabs followed by rodent and boot socks samples had higher occurrence compared to other samples during the entire study period. However, no significant difference could be observed in week wise occurrence between toilet, boot socks and rodent samples.

With respect to week wise occurrence of ESBL *E. coli*, a significantly higher ($P \leq 0.01$) occurrence was observed in human toilet swabs, boot socks and rodent samples during week 13 to week 20 as compared to other weeks and in feed and human hands swabs. None of the water samples revealed presence of ESBL *E. coli* during the entire period of study.

A significant difference ($P > 0.05$) in week wise occurrence of sulfonamide resistant *E. coli* could be observed with higher occurrence during all the weeks under the study period in human toilet, boot socks and rodents. However, the occurrence of sulfonamide resistant *E. coli* in feed, water and hand swabs was found to be inconsistent as the occurrence could not show any definite trends during the study period.

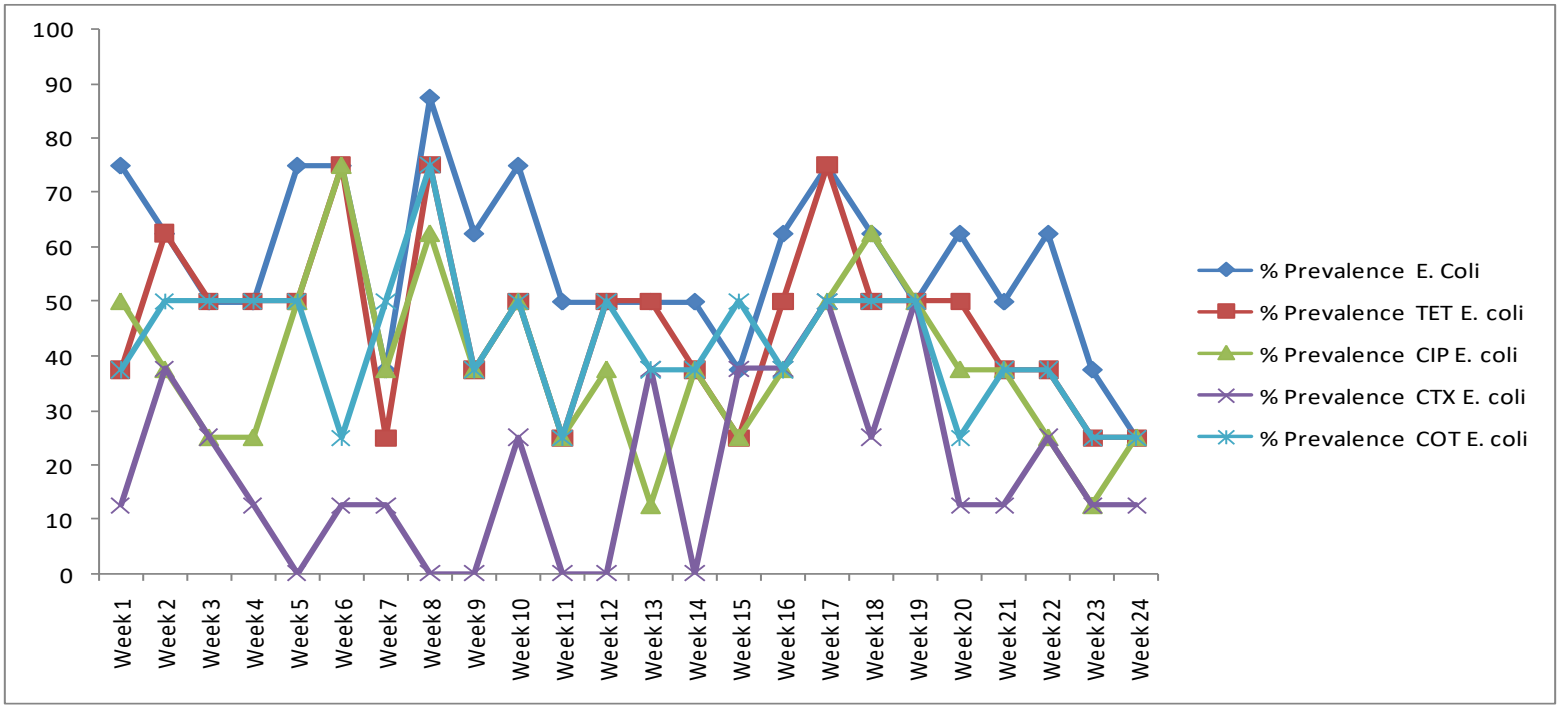


Fig. 4.4: Occurrence of *E. coli* and AMR *E. coli* in environment, human and rodent samples during the period of study (Week 1 to Week 24)

Table 4.8: Percent week wise occurrence of *E. coli* in environmental, human and rodent samples collected from the goat farm

Week	Feed* (n=48)	Water* (n=48)	Hand swab (n=24)	Human toilet (n=24)	Boot sock (n=24)	Rodent (n=24)	Week wise occurrence		
	%	%	%	%	%	%	T	P	%
1	0	100	100	100	100	100	8	6	**75**
2	0	50	100	100	100	100	8	5	**62.5**
3	50	0	100	100	0	100	8	4	**50**
4	0	50	0	100	100	100	8	4	**50**
5	50	50	100	100	100	100	8	6	**75**
6	50	50	100	100	100	100	8	6	**75**
7	0	0	100	0	100	100	8	3	**37.5**
8	100	50	100	100	100	100	8	7	**87.5**
9	50	50	100	100	100	0	8	5	**62.5**
10	100	50	0	100	100	100	8	6	**75**
11	50	50	0	100	0	100	8	4	**50**
12	0	100	0	100	0	100	8	4	**50**
13	50	50	0	100	100	0	8	4	**50**
14	50	50	0	100	100	0	8	4	**50**
15	50	0	0	100	100	0	8	3	**37.5**
16	50	0	100	100	100	100	8	5	**62.5**
17	50	100	0	100	100	100	8	6	**75**
18	50	50	0	100	100	100	8	5	**62.5**
19	0	0	100	100	100	100	8	4	**50**
20	50	0	100	100	100	100	8	5	**62.5**
21	0	50	0	100	100	100	8	4	**50**
22	50	50	0	100	100	100	8	5	**62.5**
23	0	0	0	100	100	100	8	3	**37.5**
24	0	0	0	0	100	100	8	2	**25**
	35.42 (17/48)	**39.58 (19/48)**	**45.83 (11/24)**	**91.66 (22/24)**	**87.50 (21/24)**	**83.33 (20/24)**	**192**	**110**	**57.29**

*Feed includes Fodder and Concentrate; Water includes Tank water and Trough water.

Table 4.9: Percent week wise occurrence of Tetracycline resistant *E. coli* in environmental, human and rodent samples collected from the goat farm

Week	Feed* (n=48)	Water* (n=48)	Hand swab (n=24)	Human toilet (n=24)	Boot sock (n=24)	Rodent (n=24)	Week wise occurrence		
	%	%	%	%	%	%	T	P	%
1	0	50	0	100	100	0	8	3	**37.50**
2	0	50	100	100	100	100	8	5	**62.50**
3	50	0	100	100	0	100	8	4	**50.00**
4	0	50	0	100	100	100	8	4	**50.00**
5	50	0	100	100	100	0	8	4	**50.00**
6	50	50	100	100	100	100	8	6	**75.00**
7	0	0	100	0	100	0	8	2	**25.00**
8	50	50	100	100	100	100	8	6	**75.00**
9	0	50	100	100	0	0	8	3	**37.50**
10	50	50	0	100	0	100	8	4	**50.00**
11	50	0	0	0	0	100	8	2	**25.00**
12	0	100	0	100	0	100	8	4	**50.00**
13	50	50	0	100	100	0	8	4	**50.00**
14	0	50	0	100	100	0	8	3	**37.50**
15	50	0	0	0	100	0	8	2	**25.00**
16	50	0	0	100	100	100	8	4	**50.00**
17	50	100	0	100	100	100	8	6	**75.00**
18	50	0	0	100	100	100	8	4	**50.00**
19	0	0	100	100	100	100	8	4	**50.00**
20	50	0	0	100	100	100	8	4	**50.00**
21	0	50	0	100	100	0	8	3	**37.50**
22	0	50	0	100	0	100	8	3	**37.50**
23	0	0	0	100	100	0	8	2	**25.00**
24	0	0	0	0	100	100	8	2	**25.00**
	25.00 (12/48)	**31.25 (15/48)**	**33.33 (8/24)**	**83.33 (20/24)**	**75.00 (18/24)**	**62.50 (15/24)**	**192**	**88**	**45.83**

*Feed includes Fodder and Concentrate; Water includes Tank water and Trough water.

Table 4.10: Percent week wise occurrence of Fluoroquinolone resistant *E. coli* in environmental, human and rodent samples collected from the goat farm

Week	Feed* (n=48)	Water* (n=48)	Hand swab (n=24)	Human toilet (n=24)	Boot sock (n=24)	Rodent (n=24)	Week wise occurrence		
	%	%	%	%	%	%	T	P	%
1	0	50	0	100	100	100	8	4	**50.00**
2	0	0	100	100	0	100	8	3	**37.50**
3	0	0	0	100	0	100	8	2	**25.00**
4	0	0	0	0	100	100	8	2	**25.00**
5	00	0	100	100	100	100	8	4	**50.00**
6	50	50	100	100	100	100	8	6	**75.00**
7	0	0	100	0	100	100	8	3	**37.50**
8	50	50	100	100	0	100	8	5	**62.50**
9	50	50	0	100	0	0	8	3	**37.50**
10	50	0	0	100	100	100	8	4	**50.00**
11	50	0	0	0	0	100	8	2	**25.00**
12	0	50	0	100	0	100	8	3	**37.50**
13	50	0	0	0	0	0	8	1	**12.50**
14	50	50	0	0	100	0	8	3	**37.50**
15	50	0	0	0	100	0	8	2	**25.00**
16	50	0	0	100	100	0	8	3	**37.50**
17	50	0	0	100	100	100	8	4	**50.00**
18	50	50	0	100	100	100	8	5	**62.50**
19	0	0	100	100	100	100	8	4	**50.00**
20	50	0	0	100	100	0	8	3	**37.50**
21	0	0	0	100	100	100	8	3	**37.50**
22	0	0	0	100	0	100	8	2	**25.00**
23	0	0	0	0	100	0	8	1	**12.50**
24	0	0	0	0	100	100	8	2	**25.00**
	25.00 (12/48)	**14.58 (7/48)**	**25.00 (6/24)**	**66.67 (16/24)**	**66.67 (16/24)**	**70.83 (17/24)**	**192**	**74**	**38.54**

*Feed includes Fodder and Concentrate; Water includes Tank water and Trough water

Table 4.11: Percent week wise occurrence of ESBL *E. coli* in environmental, human and rodent samples collected from the goat farm

Week	Feed* (n=48)	Water* (n=48)	Hand swab (n=24)	Human toilet (n=24)	Boot sock (n=24)	Rodent (n=24)	Week wise occurrence		
	%	%	%	%	%	%	T	P	%
1	0	0	0	100	0	0	8	1	**12.50**
2	0	0	0	100	100	100	8	3	**37.50**
3	0	0	0	100	0	100	8	2	**25.00**
4	0	0	0	0	0	100	8	1	**12.50**
5	0	0	0	0	0	0	8	0	**0**
6	0	0	0	0	0	100	8	1	**12.50**
7	0	0	0	0	100	0	8	1	**12.50**
8	0	0	0	0	0	0	8	0	**0**
9	0	0	0	0	0	0	8	0	**0**
10	0	0	0	0	100	100	8	2	**25.00**
11	0	0	0	0	0	0	8	0	**0**
12	0	0	0	0	0	0	8	0	**0**
13	100	0	0	100	100	0	8	3	**37.50**
14	0	0	0	0	0	0	8	0	**0**
15	100	0	0	100	100	0	8	3	**37.50**
16	100	0	0	100	100	0	8	3	**37.50**
17	100	0	0	100	100	100	8	4	**50.00**
18	0	0	0	0	100	100	8	2	**25.00**
19	0	0	100	100	100	100	8	4	**50.00**
20	0	0	0	0	0	100	8	1	**12.50**
21	0	0	0	100	0	0	8	1	**12.50**
22	0	0	0	100	0	100	8	2	**25.00**
23	0	0	0	0	100	0	8	1	**12.50**
24	0	0	0	0	0	100	8	1	**12.50**
	16.67 (4/48)	**0 (0/48)**	**4.17 (1/24)**	**41.67 (10/24)**	**41.67 (10/24)**	**45.83 (11/24)**	**192**	**36**	**18.75**

*Feed includes Fodder and Concentrate; Water includes Tank water and Trough water

Table 4.12: Percent week wise occurrence of Sulfonamide resistant *E. coli* in environmental, human and rodent samples collected from the goat farm

Week	Feed* (n=48)	Water* (n=48)	Hand swab (n=24)	Human toilet (n=24)	Boot sock (n=24)	Rodent (n=24)	Week wise occurrence		
	%	%	%	%	%	%	T	P	%
1	0	50	0	100	100	0	8	3	**37.50**
2	100	0	0	100	100	100	8	4	**50.00**
3	100	0	100	100	100	0	8	4	**50.00**
4	0	50	0	100	100	100	8	4	**50.00**
5	100	0	100	100	100	0	8	4	**50.00**
6	100	0	0	0	100	0	8	2	**25.00**
7	100	0	100	100	0	100	8	4	**50.00**
8	100	50	100	100	100	100	8	6	**75.00**
9	0	50	100	100	0	0	8	3	**37.50**
10	0	50	0	100	100	100	8	4	**50.00**
11	100	0	0	0	0	100	8	2	**25.00**
12	0	100	0	100	0	100	8	4	**50.00**
13	0	50	0	100	100	0	8	3	**37.50**
14	0	50	0	100	100	0	8	3	**37.50**
15	0	0	100	100	100	100	8	4	**50.00**
16	100	0	0	100	0	100	8	3	**37.50**
17	0	50	100	0	100	100	8	4	**50.00**
18	100	0	0	100	100	100	8	4	**50.00**
19	0	0	100	100	100	100	8	4	**50.00**
20	100	0	0	0	0	100	8	2	**25.00**
21	0	50	0	100	100	0	8	3	**37.50**
22	0	50	0	100	0	100	8	3	**37.50**
23	0	0	0	100	100	0	8	2	**25.00**
24	0	0	0	0	100	100	8	2	**25.00**
	41.67 (10/48)	**25.00 (12/48)**	**33.33 (8/24)**	**79.17 (19/24)**	**70.83 (17/24)**	**62.50 (15/24)**	**192**	**81**	**42.19**

4.5 Occurrence of genes encoding Antimicrobial resistance

A total of 913 *E. coli* isolates (Kids-455, Does- 209, milk-34, Feed- 31, Water-24, hand -17, human toilet- 50, boot socks- 48 and rodent- 45) obtained in this study were screened for the presence of genes encoding resistance to tetracycline (*tet*A and *tet*M), fluoroquinolones (*qnr*A, *qnr*B, *oqx*AB, *qep*A, *qnr*C, *qnr*D, *qnr*S and *aac*), Extended spectrum β lacatamase ($bla_{\text{CTX-M}}$, bla_{TEM}, bla_{SHV} and bla_{OXA}), sulfonamide resistance (*sul1* and *sul2*) and colistin resistance (*mcr1, mcr2, mcr3, mcr4* and *mcr5*)

4.5.1 Overall occurrence of AMR genes in goat production system

The occurrence of genes encoding tetracycline, fluoroquinolone, sulfonamide, ESBL and colistin resistance in *E. coli* isolated from different samples of goat production system are presented in Table 4.13 and 4.14 and graphically represented in Fig. 4.5 (a,b,c,d).

4.5.1.1 Tetracycline resistance genes (TRG)

The overall occurrence of tetracycline resistance gene in the *E. coli* isolates from the goat production system was 67.25 per cent (614/913), with majority of the isolates carrying *tet*A gene (613/913) and none of the isolate carried *tet*M gene (Plate 4.2). Among the various *E. coli* isolates, highest occurrence tetracycline resistant gene was recorded in water (94.10%), followed by isolates from feed, human samples (hand and toilet), boot socks, rodent, does, milk and kids. The isolates from various environmental samples *viz*., boot socks, feed, water, human samples (toilet and hand swabs) and rodent

samples revealed higher occurrence of TRG (71 %) as compared to host related samples (29%) (Fig. 4.5a)

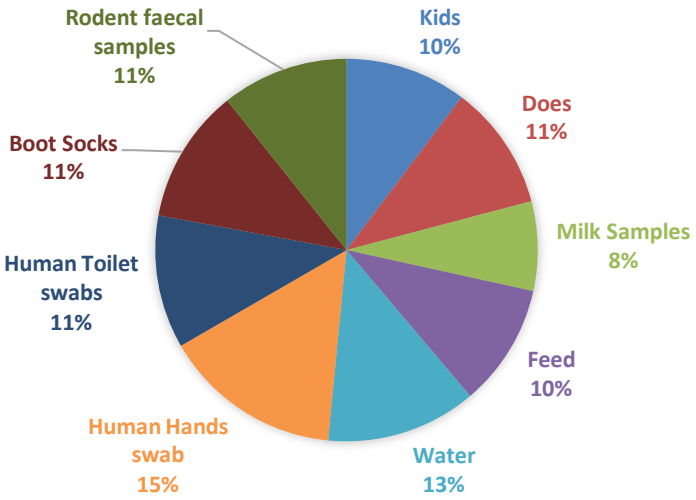
4.5.1.2 Fluoroquinolone resistance genes (FRG)

Among the 8 genes which encodes resistance to fluoroquinolones evaluated in this study, it was observed that 64.84 per cent (592/913) isolates carried *qnr*B gene, followed by *qnr*S (30.66%), *qep*A (8.76%), *oqx*AB (6.79%), *qnr*A (1.31%), *aac(6´)-Ib-cr* (0.438 %) and *qnr*D (0.328%) either alone or in combination. None of the isolates in the present study carried *qnr*C gene (Plate 4.3 & 4.4). The presence of *qnr*A, *qnr*D and *aac(6´)-Ib-cr* genes were not observed in isolates from milk, feed, human samples, boot socks and rodent samples and in similar way *oqx*AB could not been detected in isolates from water sample and *qep*A gene in isolates from hand swabs. A similar trend was established with respect to the presence of various genes encoding fluoroquinolone resistance with about 69 per cent of the isolates from various environmental samples *viz*., boot socks, feed, water, human samples (toilet and hand swabs) and rodent samples carrying either one or the other FRG and only 31 per cent of the host related sample having FRG (Fig. 4.5b)

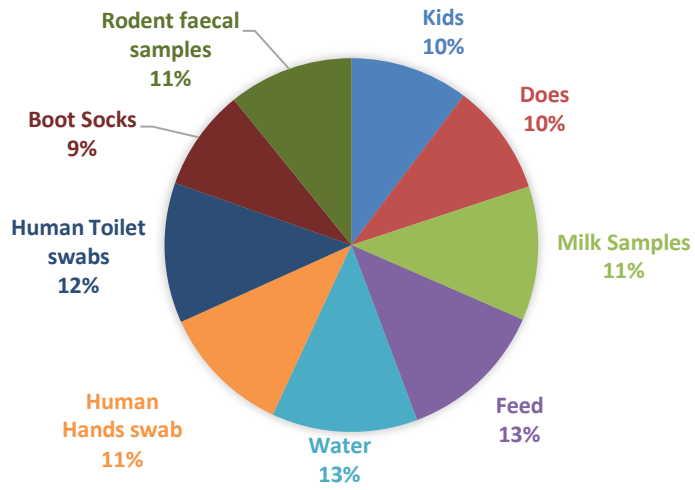
Table 4.13: Overall occurrence of gene encoding tetracycline and fluoroquinolone resistance in *E. coli* isolated from various samples in goat production system

Gene		Kids (n=455)	Does (n=209)	Milk (n=34)	Feed (n=31)	Water (n=24)	HS (n=17)	HT (n=50)	BS (n=48)	Rodent (n=45)	Total
TRG	*tetA*	291 (63.95)	138 (66.03)	16 (47.04)	20 (64.51)	19 (79.17)	16 (94.10)	35 (70.00)	34 (70.80)	30 (66.67)	613
	tetM	0	0	0	0	0	0	0	0	0	0
FRG	*qnRA*	8 (1.76)	3 (1.43)	0	0	1 (4.17)	0	0	0	0	12
	qnRB	294 (64.61)	141 (67.46)	16 (47.06)	20 (64.51)	15 (62.50)	13 (76.47)	35 (70.00)	30 (62.50)	28 (62.22)	578
	oqxAB	28 (6.15)	11 (5.26)	6 (17.65)	0	4 (16.67)	3 (17.65)	4 (8.00)	3 (6.25)	3 (6.67)	62
	qePA	41 (9.01)	14 (6.71)	6 (17.65)	5 (16.13)	4 (16.67)	0	3 (6.00)	2 (4.17)	5 (11.11)	80
	qnRC	0	0	0	0	0	0	0	0	0	0
	qnRD	1 (0.22)	0	2 (5.88)	0	0	0	0	0	0	3
	qnRS	135 (29.67)	50 (23.92)	12 (35.29)	17 (54.84)	9 (37.50)	5 (29.41)	24 (48.00)	11 (22.90)	17 (37.78)	280
	aac	1 (0.22)	1 (0.48)	1 (2.94)	1 (3.22)	0	0	3 (6.00)	0	0	4

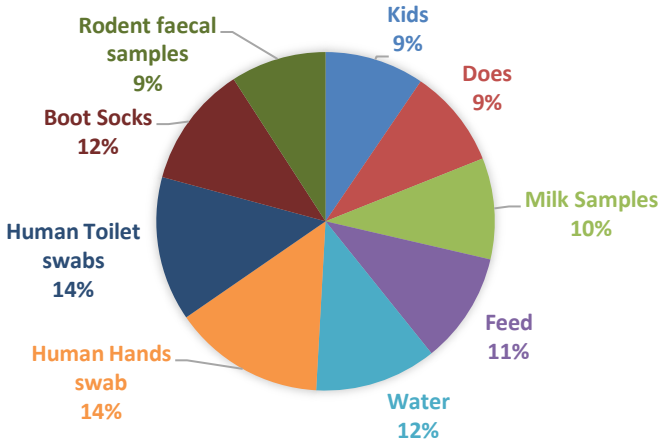
Note: HS- human hand swab, HT- human toilet swab, BS- Boot socks



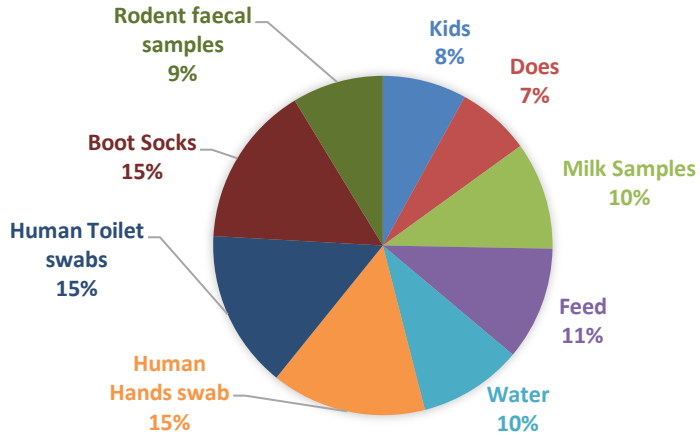
a) Tetracycline resistant genes



b) Fluoroquinolone resistant genes



c) Sulfonamide resistant genes



d) ESBL resistant genes

Fig. 4.5: Overall occurrence of antibiotic resistant genes in *E. coli* isolated from different samples of goat production system

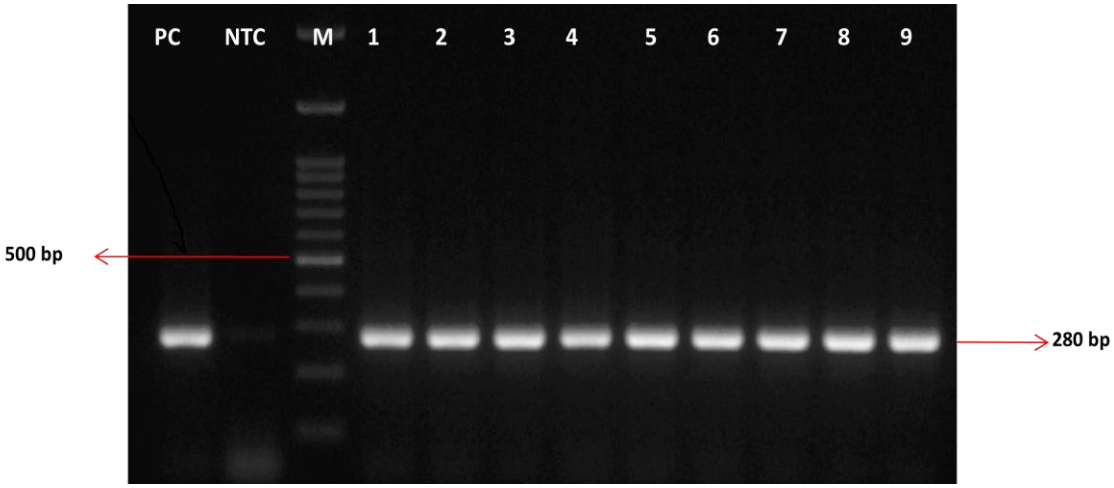


Plate 4.2: Amplification of tetracycline resistant gene (*tet*A- 280 bp) in *E. coli* isolated from goat production system

PC: Positive control (*E. coli isolate* GF6K7), NTC: Non-template control, M: 100 bp ladder; Lane 1-9: *E. coli* isolates positive for *tetM* gene from different samples from goat production system

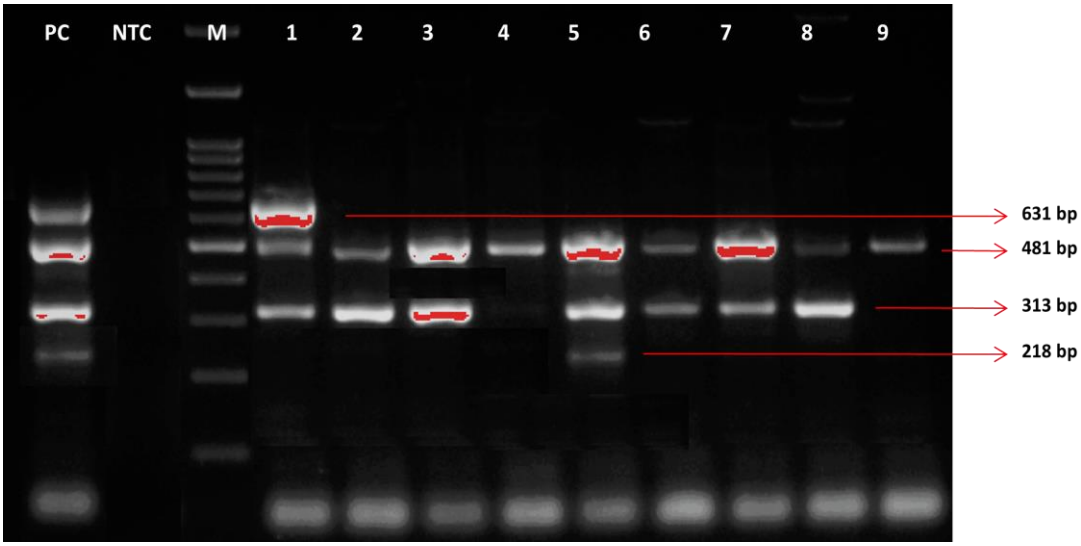


Plate 4.3: Multiplex 1: Amplification of genes encoding quinolone resistance (*qnr*A-631 bp, *qnr*B- 481 bp, *Oqx*AB- 313 bp and *qep*A- *218 bp*) in *E. coli* isolates

PC: Positive control (*E. coli* isolate VMC728), NTC: Non-template control, M: 100 bp ladder; Lane 1: *E. coli* isolate positive for *qnr*A, *qnr*B and *Oqx*AB (*E. coli* isolate GF9K7); Lane 2, 3, 6 & 7: *E. coli* isolates positive for *qnr*B and *Oqx*AB (*E. coli* isolate GF8K12); Lane 4 & 9: *E. coli* isolate positive for only *qnr*B (GF8M1, GF8M2) from different samples from goat production system

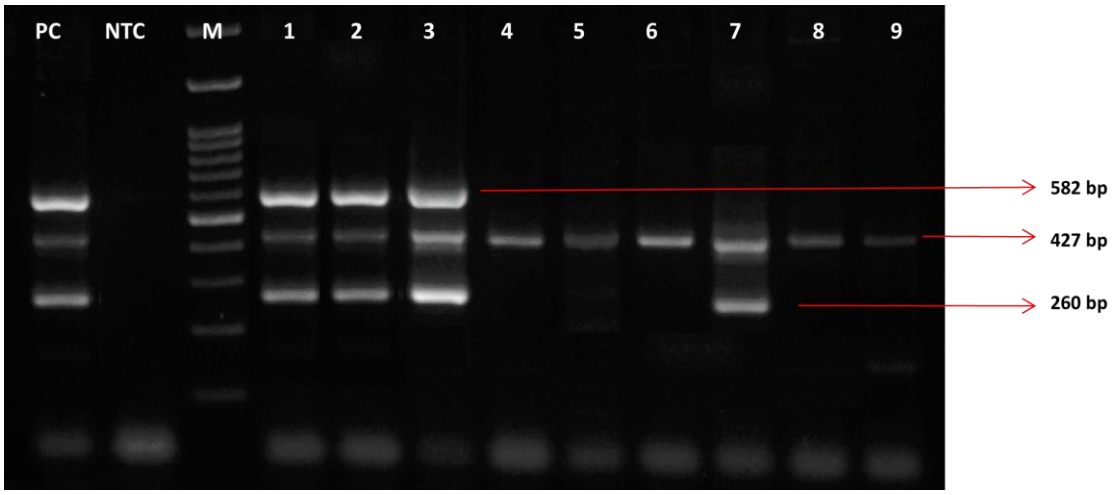


Plate 4.4: Multiplex 2: Amplification of genes encoding quinolone resistance (*qnr*D-582 bp, *qnr*S- 427 bp and *aac(6')-Ib-cr*- 260 bp) in *E. coli* isolates

PC: Positive control (*E. coli* isolate VMC791), NTC: Non-template control, M: 100 bp ladder; Lane 1, 2 & 3: *E. coli* isolate positive for *qnr*D, *qnr*S and *aac(6')-Ib-cr*; Lane 4, 5, 6, 8 & 9: *E. coli* isolates positive for *only qnr*S; Lane 7: *E. coli* isolate positive for *qnr*S and *aac(6')-Ib-cr* from different samples from goat production system

4.5.1.3 Sulfonamide resistance gene (SRG)

In the present study it was evident that among the 2 genes tested, majority of the isolates carried *sul2* gene (53.01% - 484/913), whereas only 7.88 per cent (72/913) isolates carried *sul1* gene, either alone or in combination (Plate 4.5).

It was observed that isolates obtained from environmental, human and rodents harbored 72 per cent of the genes encoding sulfonamide resistance as compared to 28 per cent of the isolates from host related samples (Fig. 4.5c).

4.5.1.4 ESBL gene

The *E. coli* isolated from various samples from goat production system revealed occurrence of either one of the four genes that encodes ESBL. Of the total 913 isolates screened, 30.34 per cent (277/913) isolates carried $bla_{\text{CTX-M}}$, followed by 27.71 per cent (253/913) carrying bla_{TEM}, 4.05 per cent (37/913) isolates carrying bla_{SHV} and only 1.10 per cent (10/913) isolates carried bla_{OXA} gene, either alone or in combination (Plate 4.6 & 4.7).

The overall occurrence of genes encoding ESBL resistance was found to be lowest among the isolates obtained from the host related samples (25%) as compared to higher presence of these genes in environmental, human and rodent isolates (75%) (Fig. 4.5d).

Table 4.14: Overall occurrence of gene encoding sulfonamide, cefotaxime (ESBL) and colistin resistance in *E. coli* isolated from various samples in goat production system

Gene		Kids (n=455)	Does (n=209)	Milk (n=34)	Feed (n=31)	Water (n=24)	Hand swab (n=17)	Human toilet (n=50)	Boot sock (n=48)	Rodent (n=45)	Total
COT	*sul1*	20 (4.40)	14 (6.70)	6 (17.65)	3 (9.67)	5 (20.83)	3 (17.61)	14 (28.00)	6 (12.50)	1 (2.22)	72 (7.88)
	sul2	243 (53.40)	106 (50.71)	14 (41.18)	17 (54.84)	12 (50.00)	12 (70.62)	28 (56.00)	28 (58.30)	24 (53.30)	484 (53.01)
ESBL	bla_{CTX-M}	134 (29.45)	52 (24.88)	9 (26.47)	9 (29.03)	9 (37.50)	6 (35.29)	24 (48.00)	25 (52.08)	18 (40.00)	277 (30.34)
	bla_{TEM}	111 (24.40)	48 (22.97)	9 (26.47)	12 (38.71)	5 (20.83)	10 (58.82)	24 (48.00)	24 (50.00)	10 (22.22)	253 (27.71)
	bla_{SHV}	14 (3.08)	3 (1.43)	6 (17.65)	2 (6.45)	3 (12.50)	2 (11.76)	4 (4.80)	3 (6.25)	0	37 (4.05)
	bla_{OXA}	2 (0.44)	2 (0.95)	1 (2.94)	1 (3.22)	0	0	2 (2.40)	1 (2.08)	0	10 (1.10)

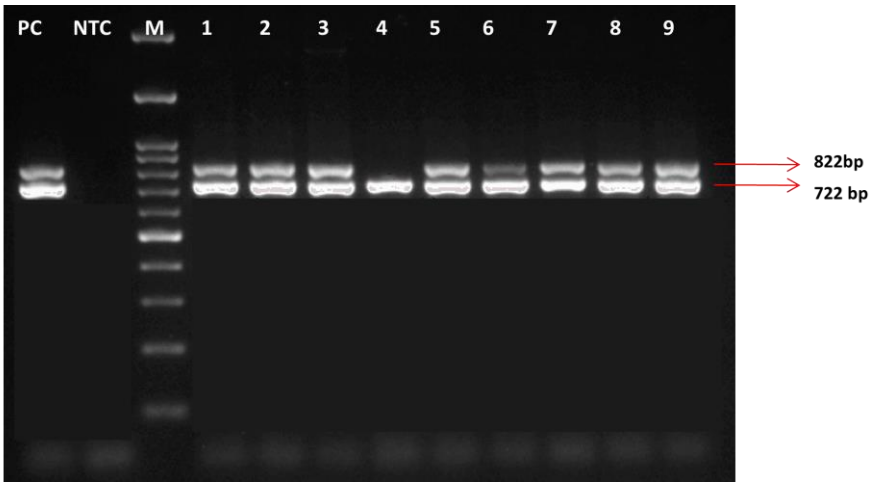


Plate 4.5: Amplification of genes encoding sulfonamide resistance (*sul1*- 822 bp and *sul2*-722 bp) in *E. coli* isolates

PC: Positive control (*E. coli* isolate VMC559), NTC: Non-template control, M: 100 bp ladder; Lane 1-3, 5-9: *E. coli* isolate positive for *sul1* and *sul2* gene; Lane 4: *E. coli* isolate positive for *only sul2* gene from different samples from goat production system

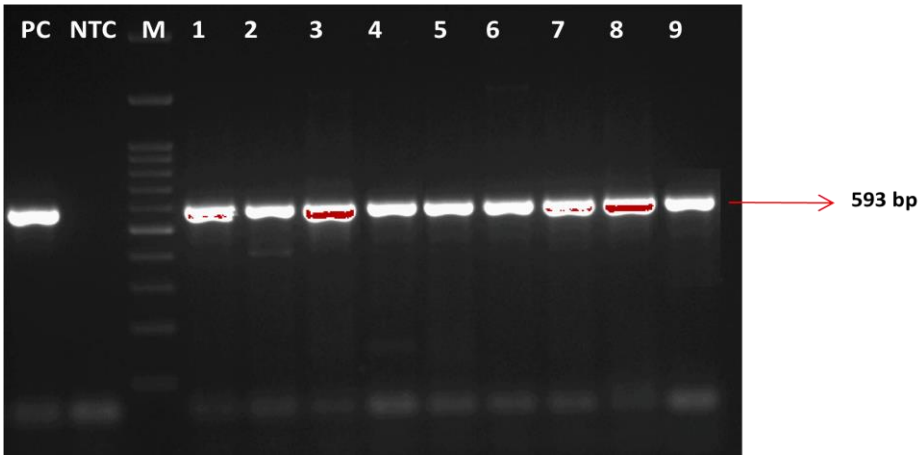


Plate 4.6: Amplification of bla_{CTX-M} (593 bp) in *E. coli* isolates

PC: Positive control (MZ636556), NTC: Non-template control, M: 100 bp ladder; Lane 1-9: *E. coli* isolate positive for bla_{CTX-M} gene from different samples from goat production system

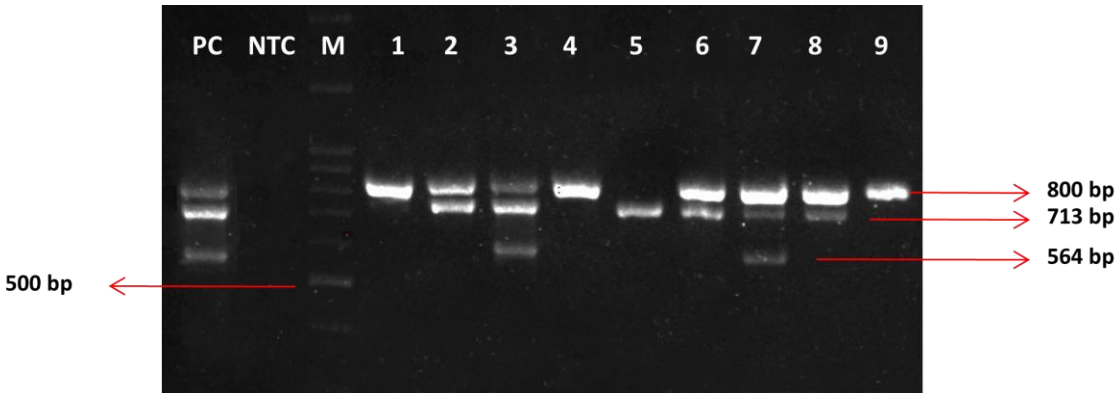


Plate 4.7: Amplification of β Lactamase genes *(bla$_{TEM}$* – 800 bp, *bla$_{SHV}$* – 713 bp *and bla$_{OXA}$* – 564 bp) in *E. coli* isolates

PC: Positive control (*bla*$_{TEM}$- MZ636559 ; *bla*$_{SHA}$- MZ636558; *bla*$_{OXA}$- MZ636557), NTC: Non-template control, M: 100 bp ladder; Lane 1, 4 & 9: *E. coli* isolate positive for only *bla$_{TEM}$*; Lane 2, 6 & 8: *E. coli* isolates positive for *bla$_{TEM}$* and *bla$_{SHV}$*; Lane 3 & 7: *E. coli* isolate positive for *bla$_{TEM}$, bla$_{SHV}$* & *bla$_{OXA}$* gene from different samples from goat production system

4.5.2 Sample wise occurrence of AMR genes

The occurrence of genes encoding resistance to tetracycline, fluoroquinolone, sulfonamide and ESBL in different samples of the goat production chain is presented graphically in Fig. 4.6 (a-i).

The presence of AMR genes in isolates from both kids and mother revealed maximum occurrence of FRG (37-38%) followed by TRG (23-24%), SRG (20-21%) and ESBL genes (18-19%). However in milk samples higher occurrence was recorded for FRG genes (40%) followed by ESBL, SRG and TRG.

The occurrence of AMR genes in isolates from environmental (feed and water) samples revealed higher presence of FRG followed by SRG, TRG and ESBL genes. However, in the human samples (hand swab, toilet swab), boot socks and in rodent samples occurrence of ESBL genes was higher (21-32 %), followed by FRG (28-39 %), SRG (18-21%) and TRG (17-23%).

The results of the AMR gene profile in various samples from the goat production system revealed the widespread occurrence of these genes in environmental, human and rodent samples as compared to host related samples indicating that these samples may play a key role in dissemination of AMR gene in the goat production system.

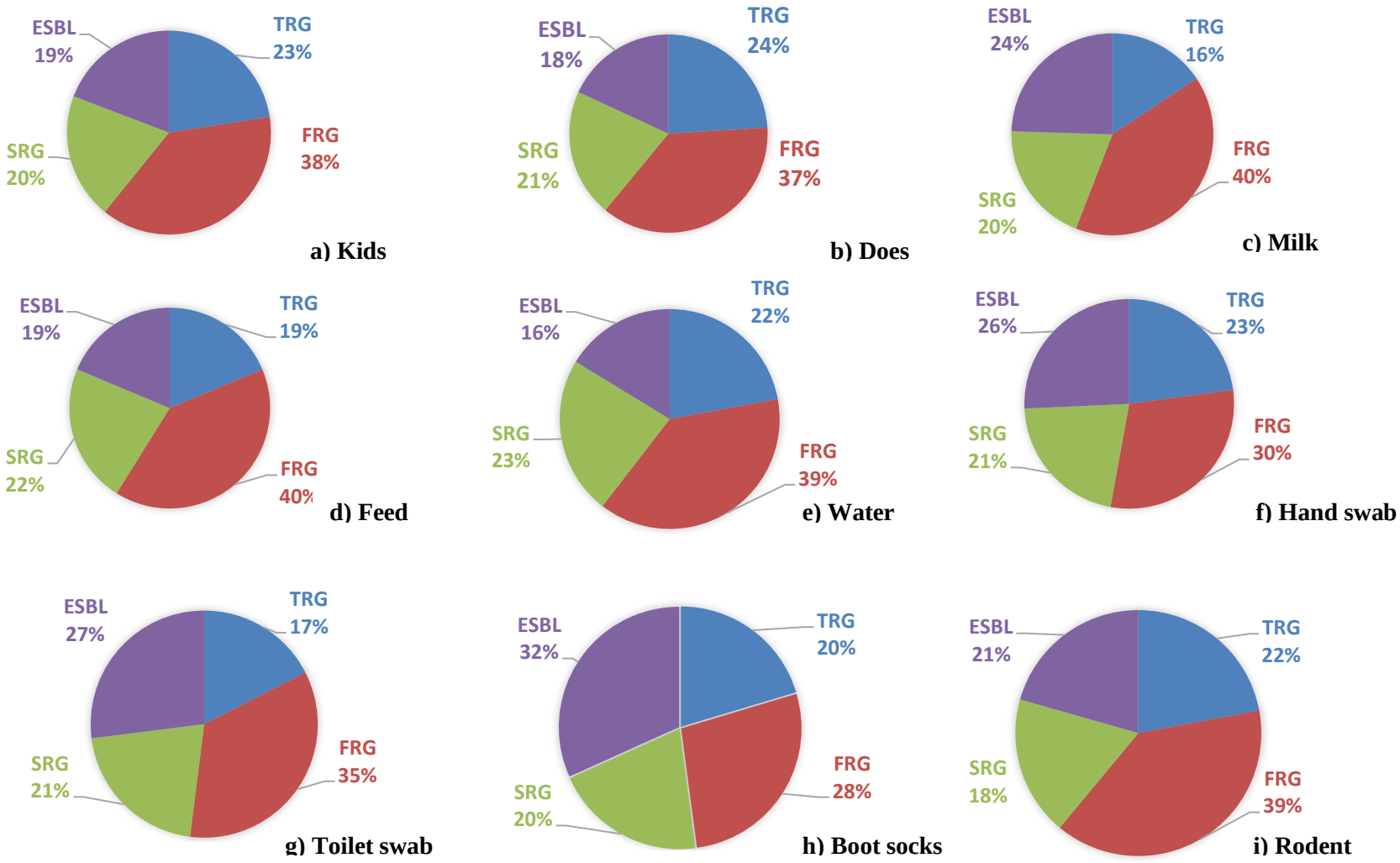


Fig. 4.6: Sample wise occurrence of different AMR genes in goat production system

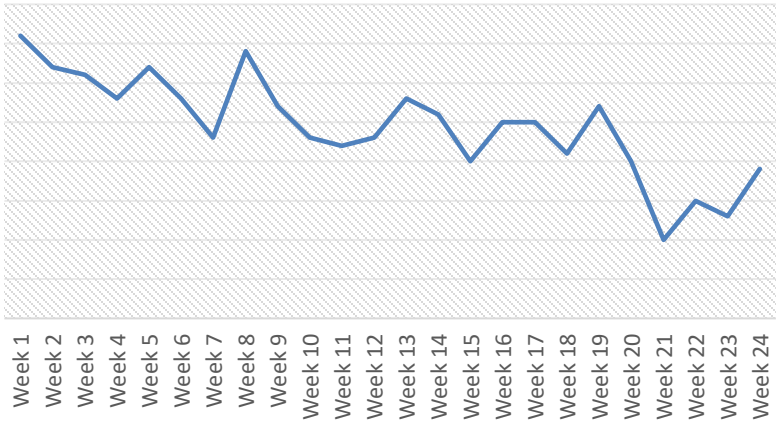
4.5.3 Week wise occurrence of AMR genes

The week wise occurrence of AMR *E. coli viz*., tetracycline, fluoroquinolone, sulfonamide and ESBL *E. coli* are graphically presented in Fig. 4.7.

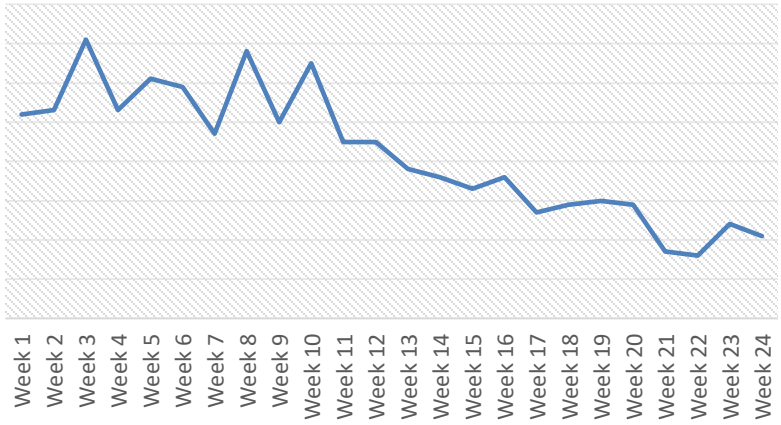
A gradual decline in week wise presence of Tetracycline genes in *E. coli* isolated of various samples in goat production system was observed in the farm over a period of 24 weeks with peak occurrence at 7-8th week. In similar lines, a gradual decrease in week wise presence of Ciprofloxacin genes was evident in the farms over a period of 24 weeks with a peak at 2nd and between 7th to 9th weeks.

With respect to occurrence of sulfonamide resistant gene in *E. coli* isolated from various farm samples during the 24 weeks of the study period, a gradual decrease was evident, whereas a peak in occurrence of genes encoding sulphonamide resistance was observed between in 4th to 6th week of the study.

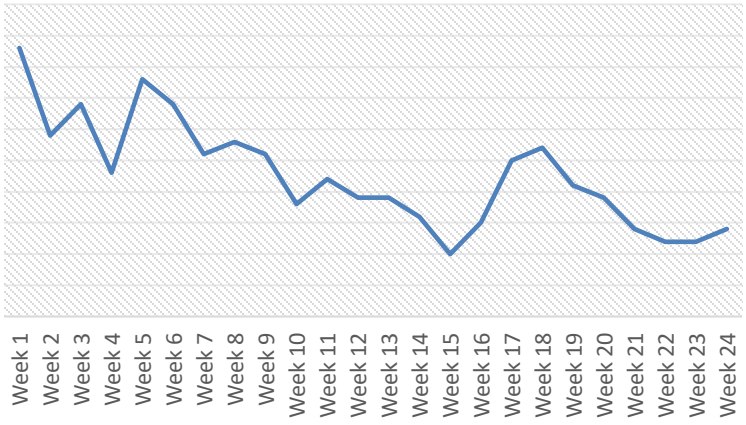
In the present study, a cyclical pattern in the week wise presence of ESBL encoding genes was evident in *E. coli* isolated from all the farm samples over a period of 24 week with a peak being observed at 2nd, 5th week and 13th weeks of the study period, however the occurrence of the gene decreased gradually towards the end of the study period.



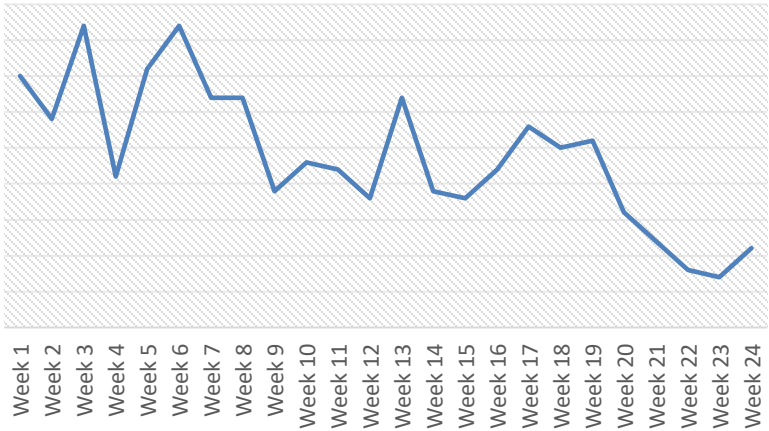
a) Tetracycline resistant gene



b) Fluoroquinolone resistant gene



c) Sulphonamide resistant gene



d) ESBL resistant gene

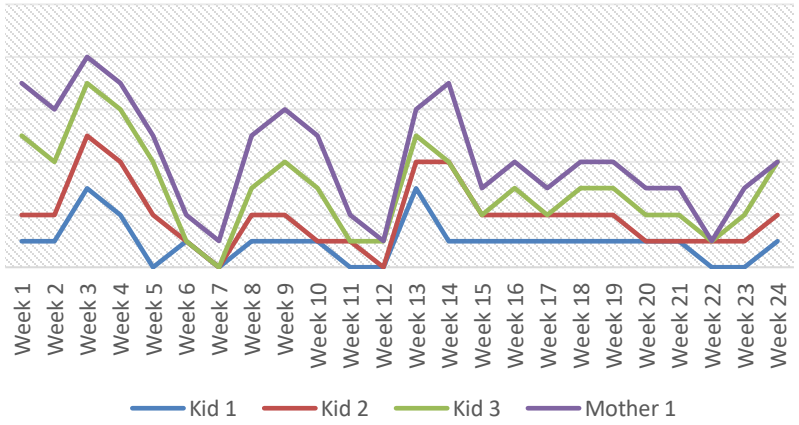
Fig. 4.7: Week wise occurrence of AMR resistance genes in goat production system

4.5.4 Group wise occurrence of AMR genes

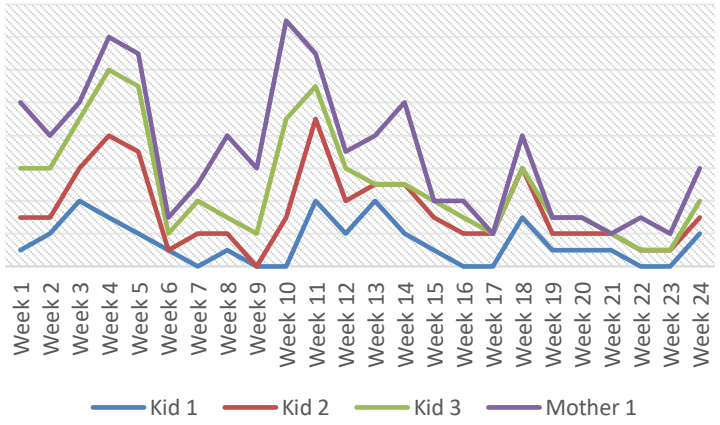
In the present study a total of 6 groups *viz*., Group I consisting of 3 kids + Does, Group II, III, IV and VI each consisting of 2 kids + does and group V consisting of 1 kid and does were evaluated to determine the occurrence of AMR within the groups and it is graphically represented in Fig. 8 to 4.13.

Irrespective of the groups it was observed that *E. coli* isolated from does had higher occurrence of all the antibiotic resistant *E. coli* as compared to their respective kids throughout the study period. In addition the occurrence of AMR genes in majority of kids in this study revealed an increase in occurrence between 7^{th} to 12^{th} weeks as compared to other weeks studied.

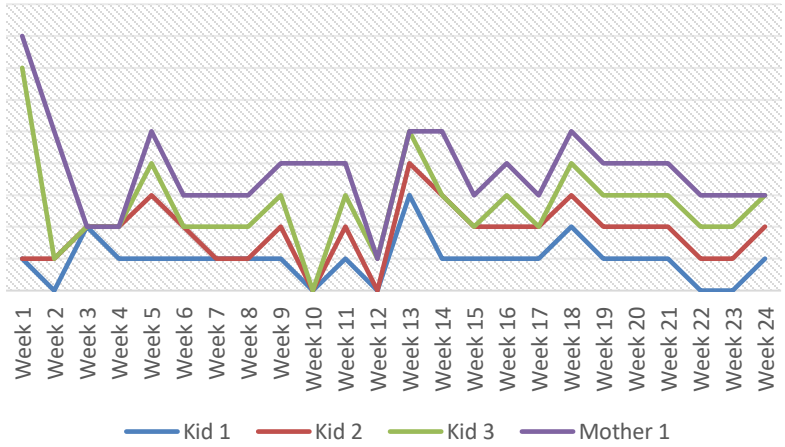
The occurrence of AMR genes showed a decline with increase in weeks in majority of the kids and does, however in some kids an increase could be observed at the end of the study period. The results of our study clearly indicated that mothers had a significant influence on the occurrence of genes encoding different antimicrobial resistance in kids during the entire study period.



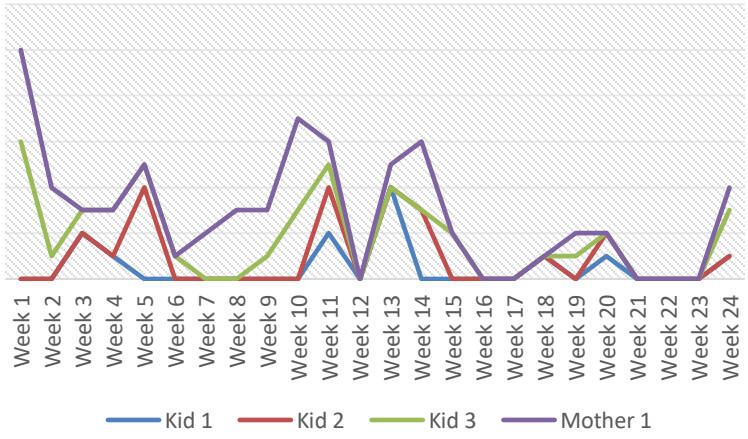
a) Tetracycline resistance gene



b) Fluoroquinolone resistance gene

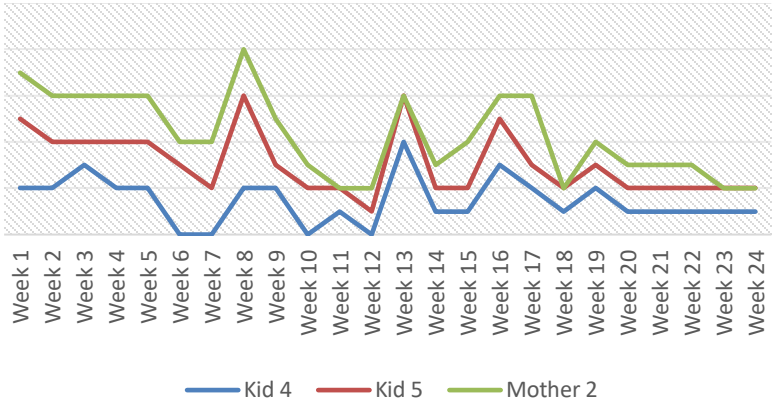


c) Sulphonamide resistance gene

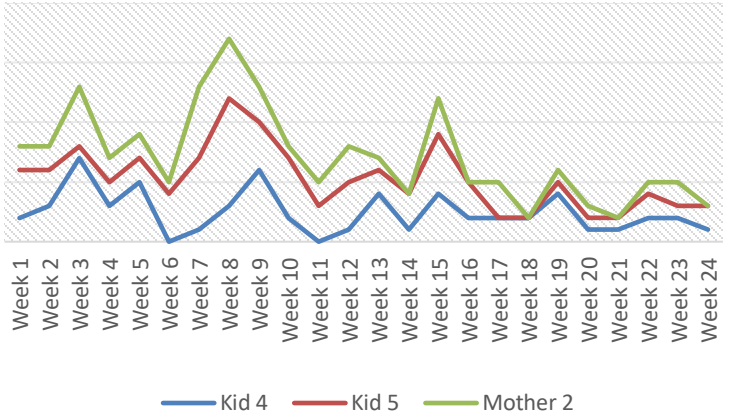


d) ESBL resistance gene

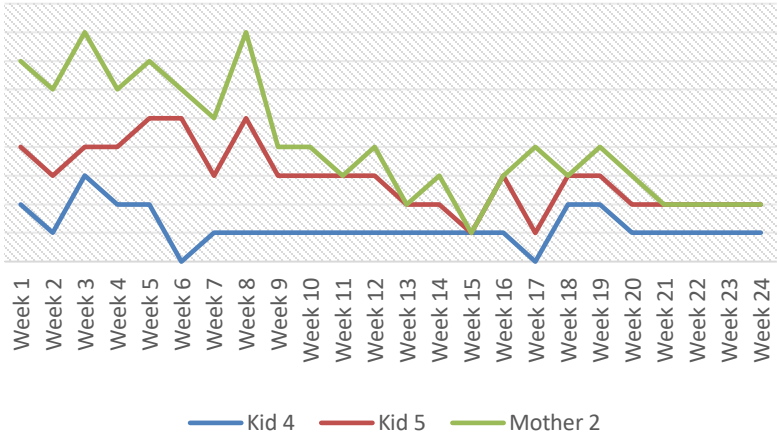
Fig. 4.8: Week wise occurrence of AMR genes in Group I (Doe no.1 and 3 of its kids)



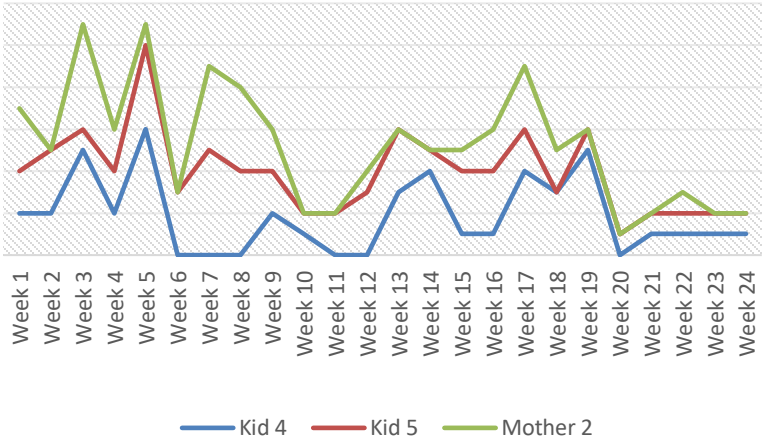
a) Tetracycline resistance gene



b) Fluoroquinolone resistance gene

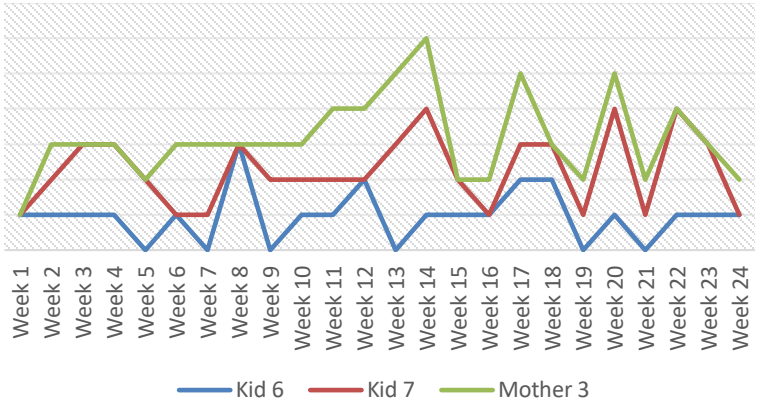


c) Sulphonamide resistance gene

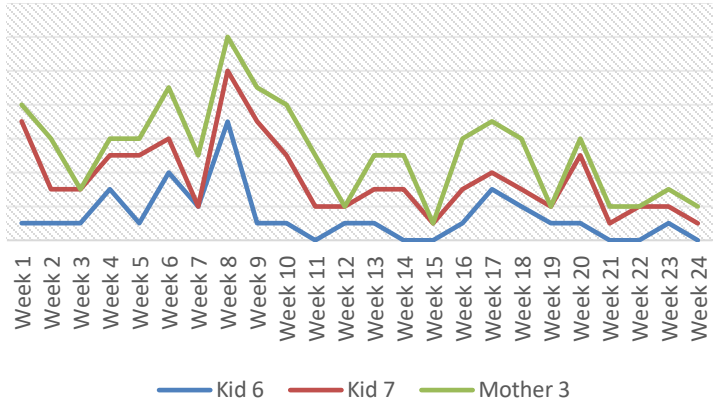


d) ESBL resistance gene

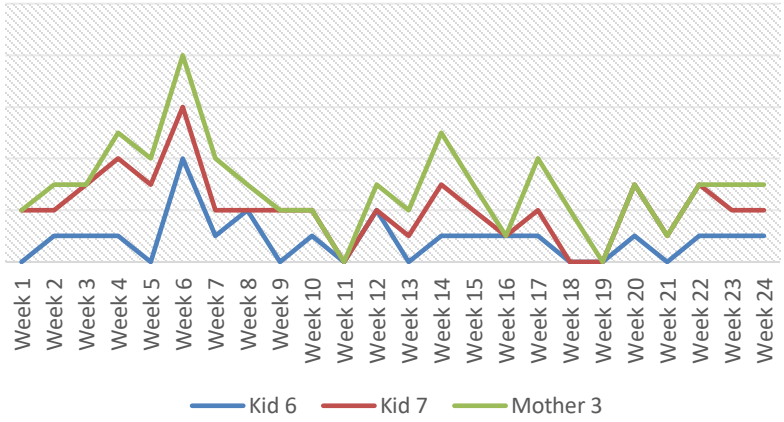
Fig. 4.9: Week wise occurrence of AMR genes in Group II (Doe no.2 and 2 of its kids)



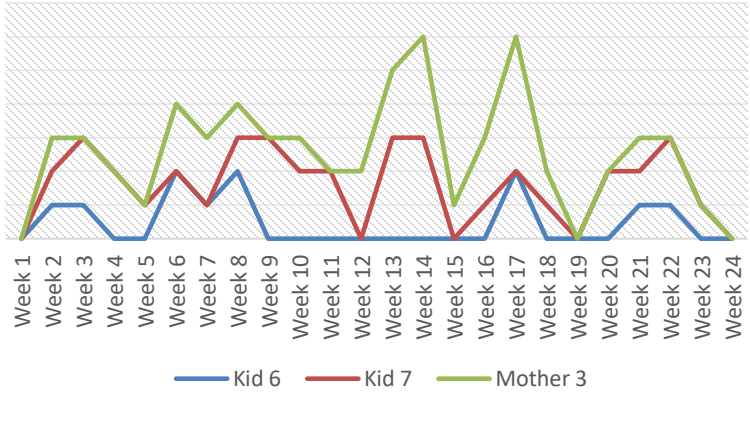
a) Tetracycline resistance gene



b) Fluoroquinolone resistance gene

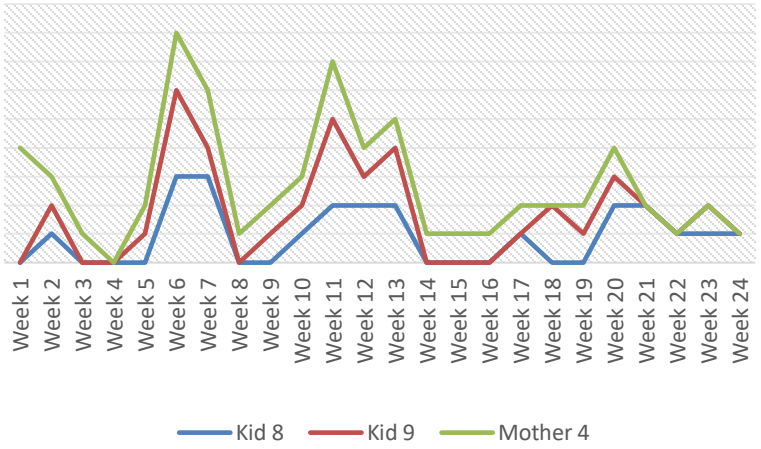


c) Sulphonamide resistance gene

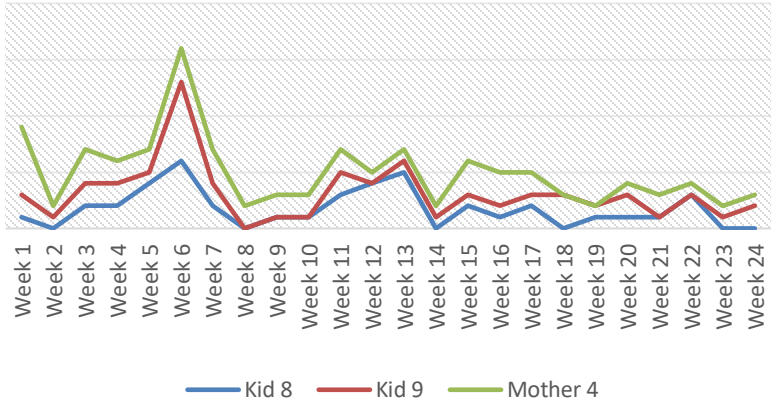


d) ESBL resistance gene

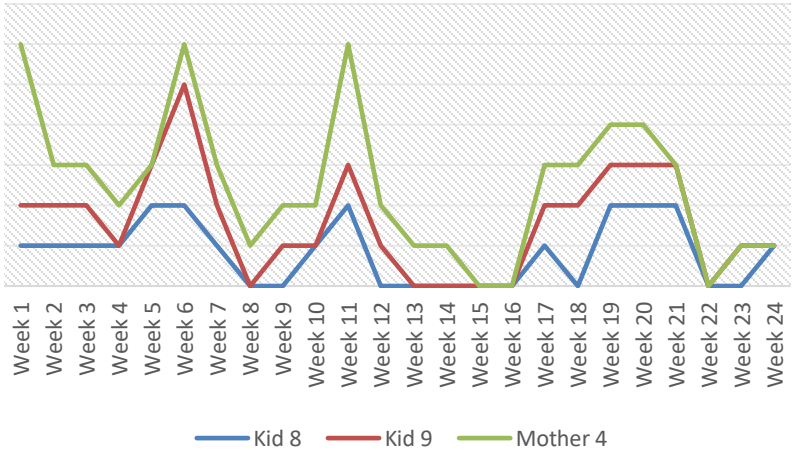
Fig. 4.10: Week wise occurrence of AMR genes in Group III (Doe no.3 and 2 of its kids)



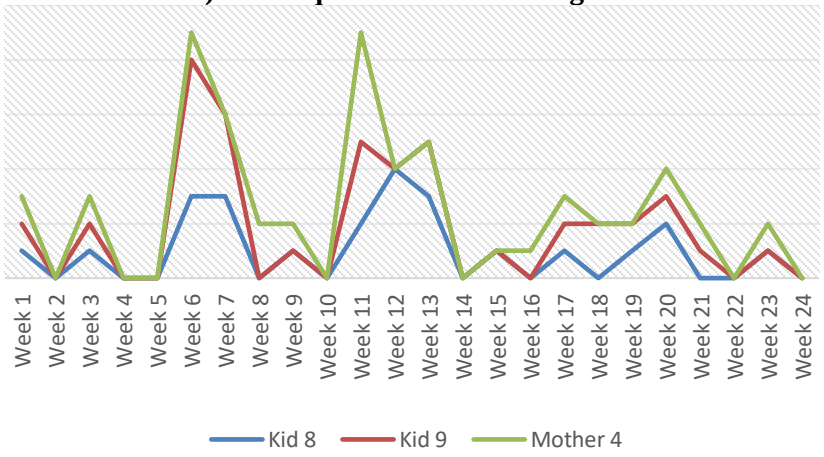
a) Tetracycline resistance gene



b) Fluoroquinolone resistance gene

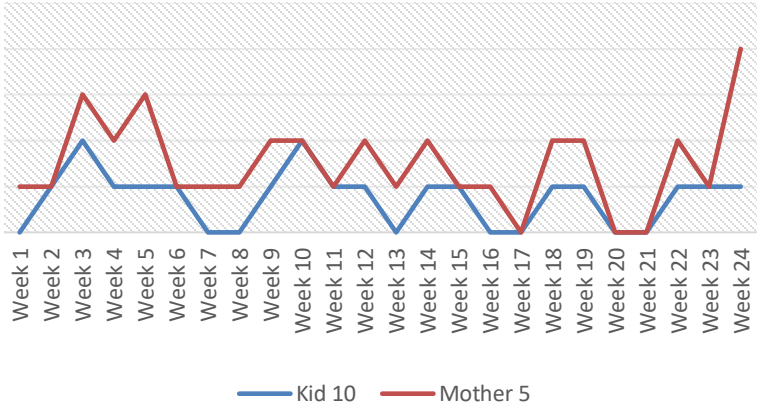


c) Sulphonamide resistance gene

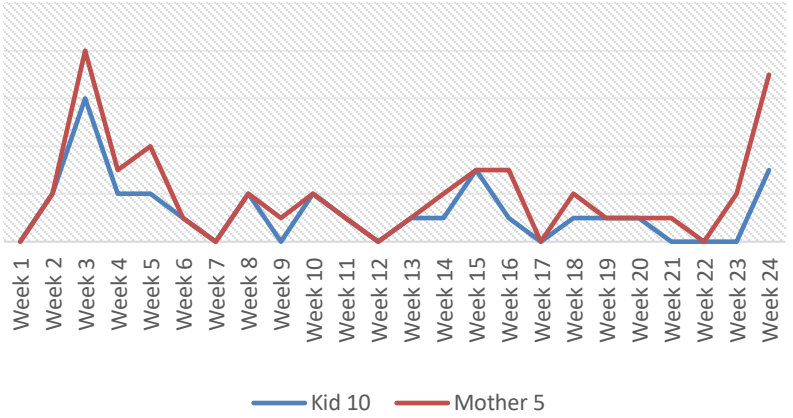


d) ESBL resistance gene

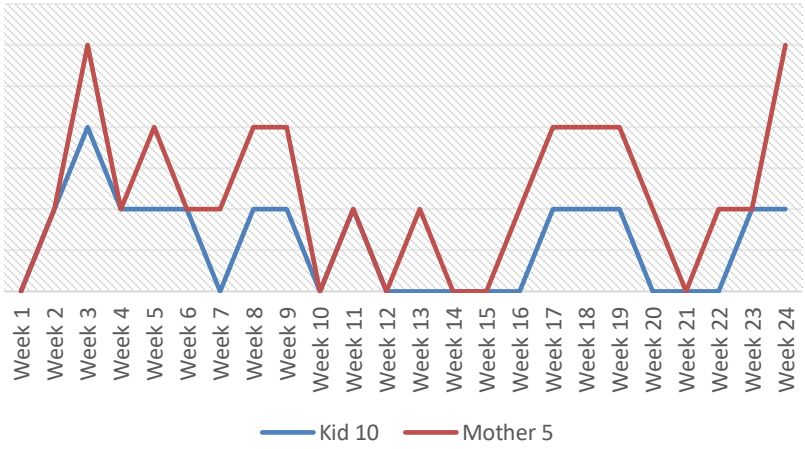
Fig. 4.11: Week wise occurrence of AMR genes in Group IV (Doe no. 4 and 2 of its kids)



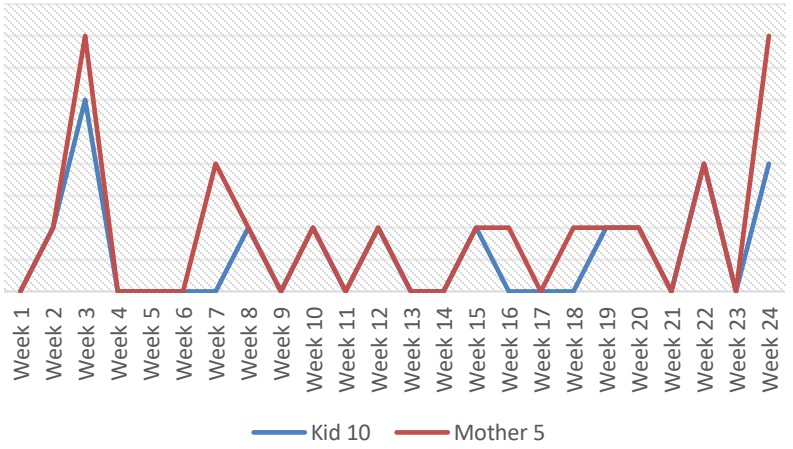
a) Tetracycline resistance gene



b) Fluoroquinolone resistance gene

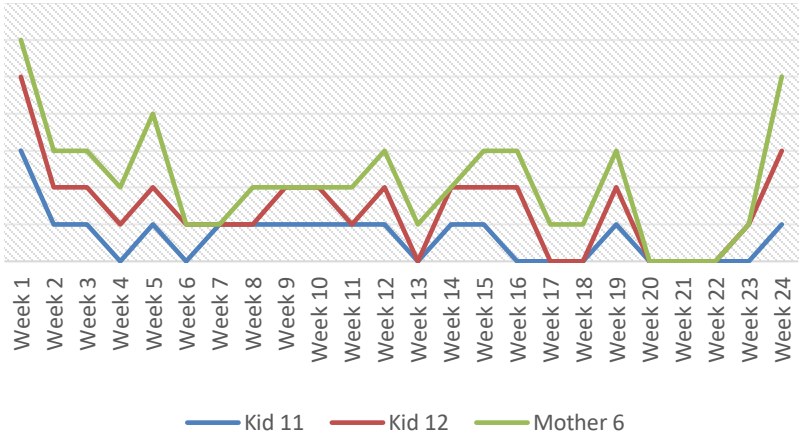


c) Sulphonamide resistance gene

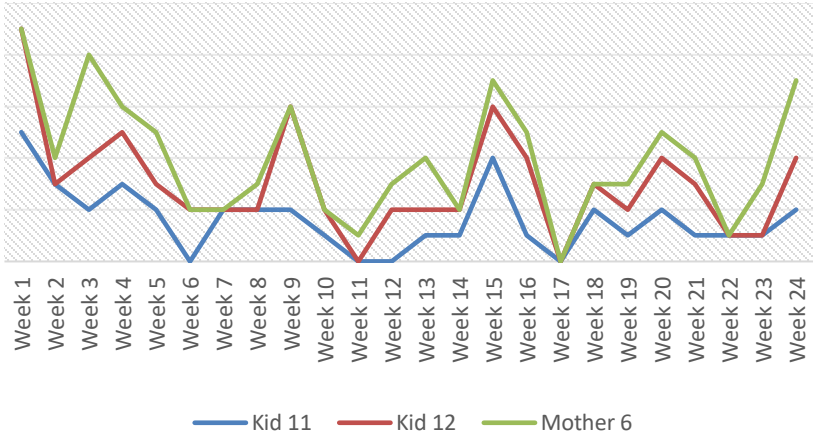


d) ESBL resistance gene

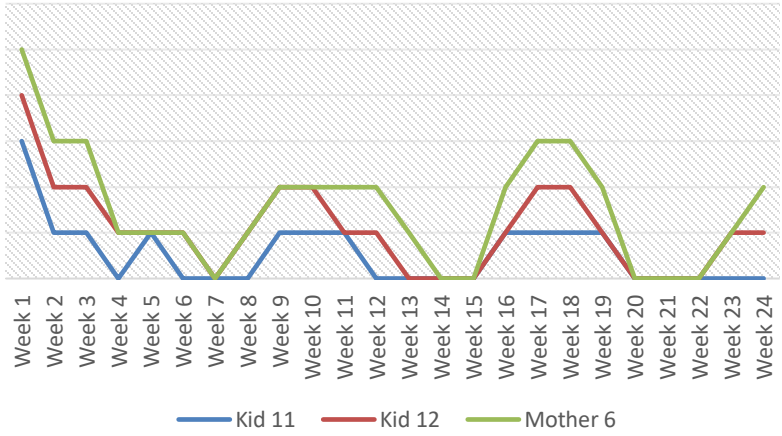
Fig. 4.12: Week wise occurrence of AMR genes in Group V (Doe no. 5 and 1 of its kid)



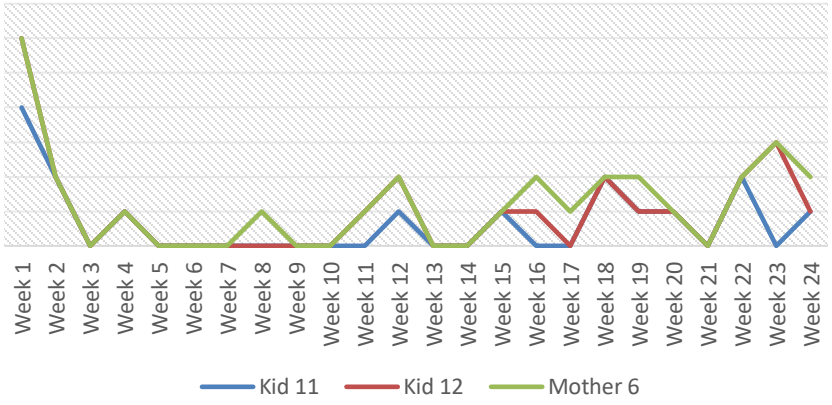
a) Tetracycline resistance gene



b) Fluoroquinolone resistance gene



c) Sulphonamide resistance gene



d) ESBL resistance gene

Fig. 4.13: Week wise occurrence of AMR genes in Group VI (Doe no. 6 and 2 of its kids)

4.3.6 Colistin resistant *E. coli*

In the present study all the 690 samples were screened in EMB supplemented with 2 mg/L of Cefotaxime and it was observed that a total of 134 samples revealed presence of colistin resistant isolates. All the samples were subject to MIC based on micro broth dilution assay as per CLSI and the results of the various isolates and their MIC values are presented in Table 4.15. The overall occurrence of colistin resistant *E. coli* with MIC value of > 2mg/L was 12.69 per cent (17/134) of the various samples tested. Among the 17 *E. coli* isolates, 10 isolates from kids, 5 isolates from does and only one isolate from milk sample had MIC value of > 2mg/L. None of the water (both tank and trough), feed (fodder and concentrate), human hand swab, boot socks and rodent samples was positive for colistin resistant *E. coli.*

All the 17 isolates were subjected to PCR for detection of genes encoding colistin resistance (*mcr1* to *mcr5*) and it was observed that none of the isolates carried *mcr1* and *mcr2*, whereas 9 isolates carried *mcr4* gene (52.94%), 7 isolates carried *mcr5* gene (41.17%) and 1 isolate carried *mcr3* gene (5.77%) either alone or in combination (Plate 4.8).

Table 4.15: MIC value of *E. coli* isolates from various samples based on Micro broth dilution method

Sample	Number of isolates	Colistin reference MIC (mg/L)									
		0.25	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**
Kids	80	1	15	41	13	3	0	0	2	2	3
Does	47	0	11	24	6	5	1	0	0	0	0
Milk	3	0	1	1	0	1	0	0	0	0	0
Feed	0	0	0	0	0	0	0	0	0	0	0
Water	0	0	0	0	0	0	0	0	0	0	0
Hand swabs	0	0	0	0	0	0	0	0	0	0	0
Toilet swabs	2	0	0	1	1	0	0	0	0	0	0
Boot socks	1	0	1	0	0	0	0	0	0	0	0
Rodent	1	0	0	1	0	0	0	0	0	0	0
Total	**134**	**1**	**28**	**68**	**20**	**9**	**1**	**0**	**2**	**2**	**3**

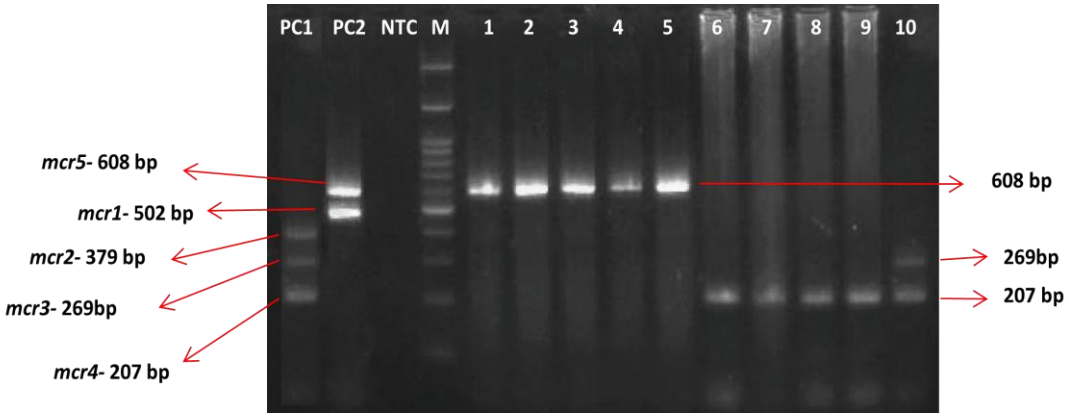


Plate 4.8: Amplification of genes encoding colistin resistance *(mcr1*- 502 bp, *mcr2*- 379 bp, *mcr3*- 269 bp, *mcr4*- 207 bp *and mcr5*- 608 bp) in *E. coli* isolates

PC1: Positive control (*mcr2*, *mcr3* and *mcr4*- MZ595143), PC2: Positive control (*mcr1 and mcr5*); NTC: Non-template control, M: 100 bp ladder; Lane 1-5: *E. coli* isolates positive for *mcr5*, Lane 6-9: *E. coli* isolates positive for *mcr4*; Lane 10: *E. coli* isolate positive for *mcr3* and *mcr4.*

Discussion

V. DISCUSSION

In the present century, microbial infections have become a major clinical threat as there are raising concerns about treatment failure due to emergence of antibiotics resistance in several commensal and pathogenic bacteria for the commonly used antibiotics. Evidence suggests that extensive and non-judicious use of antibiotics in agriculture and animal husbandry as one of the major factor contributing to the spread of this antibiotic resistance in different compartments of the ecosystem. In case of animals, antibiotics are used for treatment and or for growth promotion, however, complete absorption of these antibiotics does not take place inside the gut, and they are excreted out of the body in the form of faeces and urine, which ultimately is used as manure. It is important to understand the shedding pattern of AMR genes in a system by testing in all the components of epidemiological triad (host, agent and environment). It is also important to understand the transmission dynamics of AMR across the species, and whether there is transfer from animal to human or vice-versa. Hence in order to understand these dynamics, it is important to study the AMR pattern over a period of time and longitudinal studies are important. The work presented in this thesis is first of its kind in India by adopting longitudinal study design.

In addition, the spread of antibiotic resistance and residues are not limited to any one ecosystem and a strong correlation has been established between persistence of AMR in soil, plants, humans and animals. AMR and antimicrobial resistance genes (ARGs) travel among all these compartments. The interplay between these different ecologies is especially important in the context of antibiotic resistance and there are multiple links

between the human, animal and environmental compartments that allow not only movement of the bacteria but also of mobile genetic elements (MGEs) and traced of the antibiotic itself. Hence, there is a need for detailed, systemic and sufficient quantitative data to understand the dynamics of different bacteria and multiple resistance determinants in multiple host and environmental compartments to make effective control strategies.

It has been documented that because of its abundance in the animal gut, its ability to acquire AMR genes and ease of cultivation, *E. coli* is often used as a sentinel to understand status and for surveillance of antimicrobial resistance in both clinical and food production system. However, AMR in both commensals and pathogenic bacteria have been extensively studied in food animals including poultry, swine and cattle, but there is paucity of information globally regarding the status of AMR in small ruminant production system as a whole encompassing the different compartments in dissemination of AMR. Hence the present study was carried out to understand the temporal shedding pattern of antimicrobial resistant *E. coli* in intensive goat production system over a period of 6 months in a single intensive goat farm located in Nelamangala taluk, Bengaluru rural district, Karnataka. The results of this study are discussed with the findings of other researchers in this section.

5.1 Antimicrobial usage in goat production system

In the present study, it was evident that both does and kids were administered antibiotics frequently and the most frequently used antibiotic was enrofloxacin followed by tetracycline (both oral and parental), Sulfonamide, combination of enrofloxacin (oral) and tetracycline (injectable) and combination of enrofloxacin (oral) and Sulfonamide

(injectable) have been used only in does (2%). The higher use of enrofloxacin in this study may be attributed to its broad spectrum of activity, easy availability, lower cost and ease of administration (oral through drinking water) (Trouchon and Lefebvre, 2016). In addition, farmers prefer to use medicines that gave them quick results, its availability, their previous experience with the drug while managing similar symptoms and advice from veterinarians (Kumar and Gupta, 2018). Sahoo (2008) reported that β-lactam, fluoroquinolones and tetracyclines were the most commonly used antibiotics in dairy cattle and poultry in Odisha, India. Similar to the findings of our study, Donkor *et al.* (2012) observed that 98 per cent of the livestock keepers used antibiotics and the major antibiotics used were penicillin, oxy-tetracycline, streptomycin, sulphonamides and tylosin in Ghana.

Gemeda *et al*. (2020) observed that the most frequently used antibiotic in small ruminant production system in Ethiopia was tetracyclines followed by aminoglycosides and trimethoprim-sulfonamides. In similar lines, Bhowmik *et al*. (2017) also recorded that the mostly used antibiotic in goat were streptomycin-penicillin, followed by sulfadimidine, Amoxicillin and a combination of gentamicin-sulfadiazine-trimethoprim for treatement of sick goats in Bangladesh. In Nepal, the most common antibiotics used in animal sectors were tetracyclines, sulfa drugs, macrolides, polymyxins, bacitracin, nitrofurans, quinolones and aminoglycosides, whereas chloramphenicol was the least antibiotic consumed in the veterinary sector (Subramanya *et al*., 2021).

Based on the observation, in the farm antibiotics and their combination were used for prophylaxis to treat animals suffering from diarrhoea, respiratory symptoms, fever

and anorexia. Similar findings have been recorded by Adesokan *et al*. (2015) in Nigeria, Gemeda *et al*. (2020) in Ethiopia, Donkor *et al*. (2012) in Ghana, Mutua *et al*. (2020) in India, who opined that antibiotics were generally administered to livestock to treat respiratory diseases, fever and in general broad spectrum antibiotics were administered. In similar lines Gruel *et al*. (2021) reported that the main causes for which antimicrobials were given were respiratory diseases in pigs (45.5%), skin diseases in cattle (41.7%), and respiratory and digestive diseases in poultry (66.7%)

In addition, it was observed that manure generated from the farm was utilized for on-farm fodder cultivation. Soil fertilization with animal manure is a widespread agricultural practice worldwide, especially in developing countries, as manure is a rich source of nutrients/ organic matter and is one of the practical approaches for management of animal by lowering the cost of its disposal (Heuer *et al*., 2011). It has been documented that quinolone have been reported to be persistent in manure with dissipation time ranging from 100 to 5800 days (Berendsen *et al*. 2018), which may result in transfer to the plant/fodder grown in such manure enriched soil mainly through water transport and passive absorption, as well as facilitates persistence of AMR bacteria in the soil by selective pressure (Wellington *et al*. 2013).

5.2 Occurrence of *E. coli* and AMR *E. coli*

5.2.1 Sample wise occurrence

5.2.1.1 *E. coli*

The overall occurrence of *E. coli* from various samples from goat production system was 81.74 per cent (564/690). Similar occurrence of *E. coli* have been reported by

Manishimwe *et al*. (2021) in dairy cattle (100.0%), Lunha *et al*. (2020) in pig farm (89 %), Sobur *et al*. (2019) in dairy farm at its environment (75 %), Akond *et al*. (2009) in broiler farm (58.6%) respectively. However, no literature regarding occurrence of *E. coli* in goat production system was available to substantiate the results of this study.

A significant difference could be observed in the occurrence of *E. coli* with higher occurrence being recorded in kids (99.65%) followed by does (97.92%), human toilet swabs (92.00%), boot socks (88.00%) and rodent samples (83.00) as compared to other samples. The higher prevalence in fecal samples from kids, does and human toilet samples may be attributed to the fact that *E. coli* is a normal commensal inhabiting the gastrointestinal tract of both humans and animals (Stacy *et al*. 2014). In line with our results, higher occurrence of *E. coli* in soil samples (95%) and lowest in water samples has been documented by Sobur *et al*. (2019) from dairy cattle farm. In addition, it has been documented that an association existed between levels of *E. coli* in soil with that of ground water, hands of workers, stored water and feed in the farm premises, suggesting that soil is one of the major reservoirs for *E. coli* (Ercumen *et al*., 2017).

The occurrence of *E. coli* in rodents (83%) in our study was similar to those reported by Nkogwe *et al*. (2011) in Trinidad and Tobago (83.8%) and Himsworth *et al*. (2015) in Canada (62.7%), but was higher than reports of Ong *et al*. (2020) in Singapore (14.8%). The results of this study provided a clear insight into the occurrence of *E. coli* in rodents, which could enhance our understanding of the local epidemiology of *E. coli* within the farm setting.

It was evident that 54.00 per cent of water sample from water trough was contaminated with *E. coli.* The presence of *E. coli* in water trough may be associated with faecal contamination from the animals and other environmental sources. Lejeune *et al*. (2001) observed that cattle water troughs served as an environmental reservoir for *E. coli* O157 and it could act as a long-term source for infection in cattle. Similar to the findings of our study Osei *et al.* (2019) identified various levels of microbial contaminants in water used in poultry farms in Ghana and reported that 91% of the water used in poultry farms had various levels of microbial contamination with one or more microorganisms including *E. coli, S. typhi, S. aureus* and coagulase-negative *Staphylococci*.

The lowest occurrence in our study was recorded in feed concentrate followed by water tank, milk and fodder samples. The lowest occurrence in feed concentrate may be attributed to heat treatment process employed during preparation of concentrate feed and the occurrence in fodder samples may be linked with the use of untreated dung from the farm as organic manure for fodder production (Mukherjee *et al*. 2004).

5.2.1.2 AMR *E. coli*

The overall occurrence of tetracycline, fluoroquinolone, ESBL and Sulfonamide resistant *E. coli* irrespective of the sample type and period of collection in the entire goat production system in the present study was 42.75, 31.25, 16.23 and 48.26 per cent, respectively. Highest occurrence of AMR *E. coli* was observed in human toilet swabs, followed by soil (boot socks), rodent samples, kids and does irrespective of the type of antibiotic assessed. To our knowledge, this study is the first of its kind to document the occurrence of AMR *E. coli* from various compartment of the goat production system and

no literature was available even in other animal production system worldwide documenting occurrence of AMR from different compartments.

In the current study, there was a history of use of tetracycline, enrofloxacin and Sulfonamide for treatment of the animals during the period of study. It is possible that bacteria isolates harboring resistance existed or persisted in the soil or in the gut of does and must have colonized the study animals. This is in concurrence with findings of Jernberg *et al*. (2007) and Jakobsson *et al*. (2010), who have opined that once the bacteria develop resistance to antibiotics, the phenotypic resistance can be detected for as long as four years even after cessation of that antibiotic use.

Most *E. coli* evaluated in this study were resistant to tetracycline followed by Sulfonamide and quinolone, which is in agreement with the commonly observed resistance patterns in *E. coli* isolated from swine, cattle, and chickens (Bryan *et al*., 2004), as well as in humans (de Vries *et al*., 2011). Similarly, higher levels of resistance toward tetracycline (93%), sulfonamide (56%) and streptomycin (53%) have been documented in fecal *E. coli* from dairy farm in Pennsylvania (Cao *et al*., 2019). The findings of this study is supported by Ji *et al*. (2012) who demonstrated that the content of tetracycline and sulfonamide antibiotics was high in the soil of the farmland near the hog, cattle and chicken farms in China.

Poulin-Laprade *et al*. (2021) observed that tetracycline-resistant and CTX-resistant *Enterobacteriaceae* colonies were frequently isolated from various samples collected form Piggery farm in Canada regardless of the husbandry practice. Manishimwe *et al*. (2021) also observed that of the *E. coli* isolated from cattle faeces, 43.5 and 25.9 per

cent were resistant to cefotaxime and quinolone as detected by plating of samples in MacConkey agar added with CTX and CIP. A Swedish study on dairy farms found that environmental samples (feed and water troughs in calving areas) collected from farms had high levels of quinolone resistant *E. coli*, indicating their role in the dissemination of these organisms within the farms (Duse *et al*., 2016). Deng *et al*. (2020) opined that poultry house litter or dust includes a complex matrix of faecal bacteria, nutrients for microbial growth such as undigested feed, and feed additives including antimicrobial agents, biocides and heavy metals and may contribute to spread and circulation of the AMR pathogens within the farm environment.

The prevalence of ESBL-producing bacteria in the present study was 16.23 per cent with higher occurrence in human, soil and rodent samples as compared to other samples. Similar occurrence of ESBL *E. coli* have been reported in swine farms (Liu *et al*. 2018), broiler farms (Apostolakos *et al*. 2019) and in dairy farm settings (Mir *et al*. 2018). Gruel *et al*. (2021) observed that extended-spectrum β-lactamase (ESBL) *E. coli* isolates were detected in 7.3 per cent of pigs, 14.7 per cent of beef cattle, and 35.3 per cent of poultry. In the present study, ESBL *E. coli* was observed in all the samples except feed concentrate, water tank and trough, despite the fact that third-generation cephalosporins have not been used in the farm under study. This indicates that this kind of resistance may have been acquired from other environmental sources such as soil and rodents on the farm (Pallecchi *et al*., 2008). Soil treated with cattle manure had been reported to harbor higher abundance of β-lactam resistant bacteria as compared to untreated soil, revealing that manure-fertilized soil could harbor more antimicrobial resistant microbes (Udikovic-Kolic *et al*. 2014).

To our knowledge, this is the first comprehensive longitudinal study indicating the occurrence of AMR *E. coli* in goat production system, clearly indicating that environmental samples (water, soil), human and rodents could play a contributing role to further spread antimicrobial resistance within the farm ecosystem.

5.2.2 Week wise prevalence

5.2.2.1 Host related samples (Milk, kid and does)

A significant difference could be observed in the tetracycline, fluoroquinolone, ESBL and Sulfonamide resistant *E. coli* between the host related samples with lower resistance being observed in milk samples followed by kids and does. However, higher resistance could be observed in host related samples between 7^{th} to 13^{th} weeks, thereafter a decrease in resistance could be observed with increase in the weeks in all the host related samples, which is supported by the findings of Ndegwa *et al.* (2019). An increase in resistance could be observed especially in kids at 7^{th} to 10^{th} week, as compared to does and this might be attributed to the effect of weaning, which is a stressful period in young animals and is often associated with diarrhea in most farm animals (Ndegwa *et al.* 2020).

In this study, higher proportions of resistant *E. coli* isolates were detected in young goat kids than in does in the same environment throughout the period of study and that resistant isolates in the goat kids remained high but decreased post weaning, reaching significantly lower levels by the age of 6 months. Our findings are similar to those reported by many other researchers (Hoyle *et al*., 2004; Edrington, 2012 and Ndegwa *et al*., 2019), who have detected higher colonization by resistant *E. coli* in young calves and

pigs that declined with age of the animals. Our findings also agree with Berge *et al.* (2005) who reported a higher prevalence of AMR in younger animals than older animals.

The higher shedding of resistant *E. coli* in kids may be attributed to the commonly reported hypothesis, that young animals have an underdeveloped gut in terms of bacterial diversity and that resistant *E. coli* is able to compete successfully due to a possible linkage between resistance genes and genes conferring selective advantage in neonatal intestines. However, as the age of the animal advances, the bacterial microbiota diversifies and increases in numbers thereby resulting in loss of competitive advantage to the resistant *E. coli*, which is being slowly eliminated from the gastrointestinal tract (Khachatryan *et al.*, 2004 and Edrington, 2012). Furthermore, with the advancing age of the young animals / kids, especially post weaning, their immune system develops. This may lead to elimination of most of the pathogens which such animals would have been exposed / encountered in the young age.

5.2.2.2 Environmental samples

A significant difference in occurrence of tetracycline, fluoroquinolone ESBL and Sulfonamide resistant *E. coli* could be observed between environmental, human and rodent samples, where in human toilet swabs followed by rodent and boot socks samples had higher occurrence compared to other samples during the entire study period.

No literature is currently available to compare the weekly occurrence of AMR *E. coli* in human and environmental samples; however, to support our findings, Pormohammad *et al.* (2019) observed that the prevalence of ciprofloxacin-resistant *E.*

coli strains isolated from humans was higher than the isolated resistant strains from animals, food, and environmental sources. The higher occurrence of AMR *E. coli* in rodent samples throughout the study period indicates their significant role in dissemination of AMR, as they have access to all the three compartments of the farm ecosystem (human, animal and environment) (Gwenzi *et al*., 2021). Ndegwa *et al*. (2019) studied the longitudinal shedding patterns and characterization of AMR *E. coli* in pastured goats and opined that colonization by bacteria resistant to antibiotics that had never been used on the farm indicated the role of environment in acquisition of resistant bacteria in goats.

5.3 Overall occurrence of genes encoding Antimicrobial resistance

In the present study all the 913 *E. coli* isolates (Kids-455, Does- 209, milk-34, Feed- 31, Water- 24, hand -17, human toilet- 50, boot socks- 48 and rodent- 45) obtained were screened for the presence of genes encoding resistance to tetracycline, fluoroquinolones Extended spectrum β lacatamase, sulfonamide and colistin. The results of the occurrence of these genes sample wise, week wise and group wise are discussed in this section.

5.3.1 Tetracycline resistant genes

In the present study all the isolates were screened for presence of gene encoding ribosomal protection proteins (*tetM*) and efflux pump proteins (*tetA*). The overall occurrence of tetracycline resistance gene in the *E. coli* isolates from the goat production system was 67.25 per cent (614/913), with majority of the isolates carrying *tetA* gene and

only one isolate from kid carried *tetM* gene. The results were in concurrence with the findings of Wang *et al*. (2014), who opined that efflux is the most common mechanism of tetracycline resistance and that efflux pumps may confer high-level tetracycline resistance. Contrary to the findings of our study Ndegwa *et al*. (2019) observed that the tetracycline resistant isolates from goat production system carried only *tetB* gene (97%) and no other genes could be detected.

5.3.2 Fluoroquinolone resistant genes

Currently, three types of plasmid-mediated quinolone resistance (PMQR) genes and their variations have been more frequently reported in various bacterial pathogens around the world. These are the quinolone resistance determinant (*qnr*) genes (*qnr*A, *qnr*B, *qnr*C, *qnR*D and *qnr*S), variant aminoglycoside acetyl transferase gene [*aac(6´)-Ib-cr*] and efflux pumps-encoding genes (*qep*A and *oqx*AB) (Hernandez *et al.* 2011). Among the 8 genes which encodes resistance to fluoroquinolones evaluated in this study, it was observed that 62.87 per cent (574/913) isolates carried *qnr*B gene, followed by *qnr*S (30.66%), *qep*A (8.76%), *oqx*AB (6.79%), *qnr*A (1.31%), *aac(6´)-Ib-cr* (0.438 %) and *qnr*D (0.328%) either alone or in combination. Several researchers have observed that *qnr*S and *qnr*B to be the major genes that encode fluoroquinolone resistance in different animal production system and their environment samples (Amador *et al*., 2019; Niero *et al*., 2018; Roderova *et al*., 2017, Xia *et al*., 2010 and Akinbami *et al*., 2018). Interestingly, the *aac(6´)-Ib-cr* gene, which has been reported as the most common PMQR gene among clinical *Enterobacteriaceae* isolates (Strahilevitz *et al.* 2009), was lowest in the present study. Shabana and Al-Enazi (2020), observed that *qnr*B and *qnr*A

were the most frequently encountered PMQR genes followed by *qnr*S gene in sheep and goat in Saudi Arabia.

5.3.3 Sulfonamide resistant genes

Sulfonamide resistance is primarily mediated by the *sul*1, *sul*2 and *sul*3 genes encoding dihydropteroate synthetase (DHPS) with a low affinity for sulfonamides (Yun *et al*., 2012). The isolates of the present study were screened for the occurrence of *sul*1 and *sul*2 genes and it was observed that majority of the isolates carried *sul2* gene (53.01%), whereas only 7.88 per cent isolates carried *sul1* gene, either alone or in combination. Similar findings that occurrence of *sul2* gene was more predominant as compared to other genes have been reported by Kong *et al*. (2019) in fattening sheep farm and Medina *et al*. (2011) in calves, lamb and goat kids.

5.3.4 ESBL encoding genes

In the present study, *E. coli* isolates were screened for presence of four genes encoding ESBL ($bla_{\text{CTX-M}}$, bla_{TEM}, bla_{SHV} and bla_{OXA}) and it was evident that majority of the isolates carried $bla_{\text{CTX-M}}$ (30.34%), followed by bla_{TEM} (27.71%), bla_{SHV} (4.05%) and bla_{OXA} (1.10%), either alone or in combination. The results are in accordance with Shafiq *et al*. (2019) who detected $bla_{\text{CTX-M}}$, bla_{TEM} and bla_{SHV} in *E. coli* isolated from cloacal and nasal swabs of swine in China. It has been documented that the majority of ESBL-producing bacteria was *E. coli* and that $bla_{\text{CTX-M}}$ was the most predominant ESBL gene detected followed by bla_{TEM} and bla_{SHV} in poultry farm (Saliu *et al.*, 2017), dairy farm (Mir *et al.* 2018), cattle abattoir (Geser *et al.* 2011) and in beef cattle (Mir *et al.* 2016).

Subramanya *et al.* (2021) observed *bla*$_{\text{CTX-M}}$ gene (77.6, 43.9, and 48 per cent) was predominant among ESBL-producing *Enterobacteriaceae* from the gut of healthy humans, their reared animals, and the environment, respectively.

Previous studies have shown that the AMR genes related to aminoglycosides, tetracyclines, sulfonamides, β-lactams, macrolides, florfenicols and quinolone were widespread in faeces and soil in the hog farm, and the detection rate of AMR genes and drug resistance were both high (Xu *et al*., 2006 and Gao *et al,* 2019).

5.4 Sample wise occurrence of AMR gene

No significant difference could be observed with respect to the prevalence of genes encoding fluoroquinolone (FRG- 37-40%), tetracycline (TRG-23-24%), Sulfonamide (SRG-20-21%) and ESBL genes (18-19%) with respect to *E. coli* isolated from kids, does, milk, feed and water. However, with respect to human samples (hand swab and toilet swab), boot socks and rodent samples a significantly higher occurrence of ESBL encoding genes followed by FRG was observed as compared to host related samples. Similarly Subramanya *et al*. (2021) observed a high proportion (57%) of ESBL-producing *Enterobacteriaceae* was found to colonize the gut of farmers involved in rearing of animals, their children and the surrounding environment. Similar occurrence of ESBL-producing *Enterobacteriaceae* in healthy human population in community setting has been reported in India (68%) (Mathai *et al*., 2002), Pakistan (52%) (Jabeen *et al*., 2005) and in China (50%) (Li *et al*., 2019).

In line with the results of the present study Jones *et al*. (2013) indicated that mice in the farm premises as a source of ciprofloxacin resistant *E. coli* in turkey fattening flocks in UK. In addition, they also opined that presence of cephalosporin resistance in fattening flocks to be associated with certain external biosecurity factors such as nearby piggery units and staff working in the farm premises.

5.5 Week wise occurrence of AMR gene

A gradual decline in week wise presence of Tetracycline, sulfonamide and fluoroquinolone resistant genes was observed in the farm over a period of 24 weeks with peak occurrence at 4-12th week. In the present study, a cyclical pattern in the week wise presence of ESBL encoding genes with a peak being observed at 2nd, 5th week and 13th weeks of the study period, however the occurrence of the genes decreased gradually towards the end of the study period.

To the best of our knowledge, this phenomenon of weekly occurrence of AMR genes in *E. coli* in goat production system have not been studied or reported by other researchers worldwide. However, it may be hypothesized that the pattern observed in this study implies that acquisition and colonization of AMR gene in *E. coli* isolates is a dynamic event and it depends mainly on the gene pool in the environment as well as the timely treatment received by the animals during the study period. It is not just enough to provide the most suitable antibiotics / antimicrobials for treating the affected hosts but the timing of treatment is also equally important (Gjini and Brito, 2016). However, the timings of administration of antibiotics / antimicrobials were not considered in the present study. In concurrence with the findings of our study Hansen *et al.* (2013) in their

longitudinal study to determine the prevalence of extended spectrum cephalosporin resistant (ESC-R) *E. coli* in pig flock, observed a decrease in ESC-R prevalence with period of time in three farms in Germany.

5.6 Group wise occurrence of AMR gene

Comparison of AMR gene occurrence between the does and their respective kids revealed that *E. coli* isolated from does had higher occurrence of all the antibiotic resistant *E. coli* as compared to their respective kids throughout the study period. In addition the occurrence of AMR genes revealed a cyclical pattern with increase and decrease during the entire period of study. This may be attributed to the impact of long term and use antibiotic treatement in the animals during the study period on the host immune system.

Ankomah and Levin (2014) evaluated the interaction between pathogen and host immunity using an explicit resource-based model, however they could not establish a clear understanding about the role of host immunity in optimal treatment of resistant infections. It has been documented that antibiotic treatment induces changes that are important to microbial regulation of host immunity *viz*., loss of bacterial ligands that are recognized by the host, alterations in the metabolites produced by the microbiota and the loss of specific bacterial signals. Changes in immunity, such as those that occur as a consequence of antibiotic treatment, result in increased susceptibility to infection by pathogens (Willing *et al*. 2011).

5.7 Colistin resistant *E. coli*

In the present work, colistin resistance in 134 *E. coli*, which revealed growth in EMB supplemented with 2 mg/L of cefotaxime, was evaluated using the recommended MIC microdilution method (CLSI, 2018). The overall occurrence of colistin resistant *E. coli* was 12.69 per cent (17/134) of the various samples tested in goat production system.

PCR for detection of genes encoding colistin resistance revealed that none of the isolates harbored *mcr*1 and *mcr2,* whereas 8 isolates carried *mcr4* gene (47.06%), 5 isolates carried *mcr3* gene (29.41%) and 4 isolates carried *mcr5* gene (23.53%). In concurrence with our findings higher prevalence of *mcr4*, *mcr3* and *mcr5* have been documented in food animals by Chen *et al*. (2017) in china, Fukuda *et al*. (2017) in Japan and Alba *et al*. (2017) in Germany.

Summary

VI. SUMMARY

A comprehensive longitudinal study to understand the shedding pattern of antimicrobial resistant *E. coli* in goat meat production system encompassing all the components *viz*., host (kids, does and milk), human (hand swabs, toilet swabs), environment (soil, water and feed) and rodents was carried out at an intensive goat farm located located in Nelamangala taluk, Bengaluru rural district, Karnataka. A total of 6 does along with 12 kids were recruited for the study and samples were collected from the host related samples, human and environment at weekly intervals for a period of 6 months and used for isolation and molecular characterization of *E. coli* and AMR *E. coli* to study the dynamics of shedding and to get insights on the possible source of AMR *E. coli* spread in the goat meat production system.

Preliminary survey of the farm and on farm observations during the study period revealed that the animals (Kids and does) were administered antibiotics frequently and Enrofloxacin was the most commonly used antibiotic, followed by tetracycline, sulfonamide and combination of enrofloxacin and tetracycline. The major reason for use of antibiotics and their combination was for prophylaxis against respiratory and gastrointestinal infections. It was also observed that milk from the does treated with antibiotics was fed to the kids without any withdrawal period. The faecal droppings of the farm were utilized for on-farm fodder cultivation and the fodder produced was used for feeding of the animals in the farms.

A total of 690 samples (Kids-288, Does- 144, Milk-66 and 24 each of fodder, concentrate, boot socks, water from tank and trough, hand swabs, toilet swabs and rodent fecal sample) were screened for presence of *E. coli* by streaking on to Eosin Methylene Blue agar (EMB) with different antibiotics. Based on biochemical and molecular characterization 913 *E. coli* isolates (Kids-455, Does- 209, milk-34, Feed- 31, Water- 24, hand -17, human toilet- 50, boot socks- 48 and rodent- 45) were used for occurrence of genes encoding AMR for major class of antibiotics.

The overall occurrence of *E. coli* from various samples from goat production system irrespective of the weeks of collection was 81.74 per cent (564/690). A significant difference ($P \leq 0.05$) could be observed in the occurrence of *E. coli* from different samples with higher occurrence being recorded in kids, does, human toilet swabs, rodent samples and boot socks as compared to other samples. The overall occurrence of tetracycline, fluoroquinolone, ESBL and Sulfonamide resistant *E. coli* irrespective of the sample type and period of collection in the entire goat production system in the present study was 42.75, 31.25, 16.23 and 48.26 per cent, respectively. A significantly highest ($P \leq 0.05$) occurrence of AMR *E. coli* was observed in human toilet swabs, followed by soil (boot socks), rodent samples, kids and does irrespective of the type of antibiotic assessed.

Analysis of week wise occurrence of AMR *E. coli* in goat production system, a significant difference ($P \leq 0.05$) could be observed in the tetracycline, fluoroquinolone, ESBL and Sulfonamide resistant *E. coli* between the host related samples with lower resistance being observed in milk samples followed by kids and does. However, higher

resistance could be observed in host related samples between 7th to 13th weeks, thereafter a decrease in resistance could be observed with increase in the weeks in all the host related samples. In similar pattern, a significant difference in occurrence of tetracycline, fluoroquinolone ESBL and Sulfonamide resistant *E. coli* could be observed between environmental, human and rodent samples, where in human toilet swabs followed by rodent and boot socks samples had higher occurrence compared to other samples during the entire study period.

All the 913 *E. coli* isolates were screened for the presence of genes encoding resistance to tetracycline, fluoroquinolones, extended spectrum β lacatamase, sulfonamide and colistin. Majority of the isolates carried *tetA* gene and highest occurrence tetracycline resistant gene was recorded in water (94.10%), followed by isolates from feed, human samples (hand and toilet), boot socks, rodent, does, milk and kids. It was observed that 62.87 per cent isolates carried *qnr*B gene, followed by *qnr*S (30.66%), *qep*A (8.76%), *oqx*AB (6.79%), *qnr*A (1.31%), *aac(6´)-Ib-cr* (0.438 %) and *qnr*D (0.328%) either alone or in combination. None of the isolates in the present study carried *qnr*C gene.

In the present study, a majority of the isolates carried *sul2* gene (53.01%), whereas only 7.88 per cent isolates carried *sul1* gene, either alone or in combination. Among the ESBL encoding genes 30.34 per cent of the isolates carried $bla_{\text{CTX-M}}$, followed by bla_{TEM} (27.71%), bla_{SHV} (4.05%) and bla_{OXA} gene (1.10%) either alone or in combination. The overall occurrence of colistin resistant *E. coli* with MIC value of > 2mg/L was 12.69 per cent and it was observed that none of the isolates carried *mcr1* and

mcr2, whereas *mcr4* (52.94%), *mcr5* gene (41.17%) and *mcr3* gene (5.77%) was observed.

Sample wise analysis of AMR gene revealed, no significant difference respect to the occurrence of genes encoding fluoroquinolone (FRG- 37-40%), tetracycline (TRG-23-24%), Sulfonamide (SRG-20-21%) and ESBL genes (18-19%) with respect to *E. coli* isolated from kids, does, milk, feed and water. However, with respect to human samples (hand swab and toilet swab), boot socks and rodent samples a significantly ($P \leq 0.05$) higher occurrence of ESBL encoding genes followed by FRG was observed as compared to host related samples.

Week wise occurrence of AMR genes in the goat production system indicated a peak increase in AMR genes between 7^{th} to 13^{th} weeks in majority of the genes, however with the increase in the study period a decline in occurrence of AMR genes was evident. Comparison of AMR gene occurrence between the does and their respective kids revealed that *E. coli* isolated from does had higher occurrence of all the antibiotic resistant *E. coli* as compared to their respective kids throughout the study period. In addition the occurrence of AMR genes revealed a cyclical pattern with increase and decrease during the entire period of study.

Based on the findings of the present study the following conclusions could be drawn with respect to shedding of AMR *E. coli* in intensive goat production system.

1. Antimicrobial usage in the farm animals was found to have influence on the occurrence of AMR *E. coli* in host related (Kids and does). However, resistance

could be observed for antibiotics that have not been used in the farm premises (3rd generation Cephalosporin, colistin), which clearly indicated that human, rodents and soil could act as a potential reservoirs of AMR in farm settings and their ability to disseminate to the other compartments in contact with them.

2. Weekly data on occurrence of AMR *E. coli* indicated that in kids an increase in resistance was observed between 7th to 13th week, which indicated that weaning phase had a significant influence and the change in diet may have also affected the pattern of occurrence. However, it is beyond the purview of this study to investigate the influence of stress of weaning and diet on the occurrence of AMR *E. coli*.

3. Throughout the study period samples from boot socks (soil), rodents and human toilet swabs were found to harbor higher levels of AMR *E. coli* indicating their potential as reservoir in dissemination within in the farm ecosystem. However, data pertaining to the genetic relatedness of these isolates as compared to isolates from host related samples were not evaluated in this study to provide clear evidences.

4. Fodder samples collected from the farm under study was also found to harbor AMR *E. coli*, which could be attributed to the application of untreated fecal materials from the animals as organic manure. However, the dynamics of AMR *E. coli* in soil environment needs further investigation before implicating its role in dissemination to the fodder.

5. AMR gene profile of the isolates from various samples indicated no significant difference with respect to genes encoding tetracycline, fluoroquinolone and Sulfonamide resistance, indicating the circulation of these genes within the animals and farm environment as majority of the genes studied were plasmid mediated. However, with respect to ESBL encoding genes environmental, rodent and human samples had significantly higher occurrence as compared to host related samples indicating their role in spread of ESBL *E. coli* to the animals since none of the animals of the study have been exposed to these antibiotics.

6. The data on occurrence AMR *E. coli* in both does and kids revealed a cyclical pattern with increase and decrease during the entire period of the study. This pattern coincides with the treatment the animals have received during this study. Hence, the influence of antibiotic therapy on modification of gut microbiome of the host and its effect on host immune system needs to be elucidated for better understanding of the phenomenon of fluctuating AMR pattern in animals.

The present longitudinal study is the first of its kind in India and the results of this study provided the first baseline information on antimicrobial use, dynamics of phenotypic and genotypic resistance to antibiotics in *E. coli* isolated from intensive goat production system. However, future research activities should be directed towards establishing the genetic relatedness of isolates from various sources within the farm premises based on various typing methods like Pulse filed gel electrophoresis (PFGE) or high resolution based typing like whole genomic sequencing (WGS) to provide better understanding of the pathways of dissemination of AMR in animal production system.

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VII. BIBLIOGRAPHY

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Abstract

VIII. ABSTRACT

A longitudinal study to understand the shedding pattern of antimicrobial resistant *E. coli* in goat meat production system was carried in an intensive goat farm in Karnataka. A total of 6 does along with 12 kids were recruited and samples were collected from the host related samples, human and environment at weekly intervals for a period of 6 months (n=690). Antibiotics were used in the farm for only for treatement and enrofloxacin was the most commonly used antibiotic (68-72%), followed by tetracycline and sulfonamide. The overall occurrence of *E. coli*, tetracycline, fluoroquinolone, Sulfonamide and ESBL resistant *E. coli* from various samples from goat production system was 81.74, 42.75, 31.25, 48.26 and 16.23 per cent, respectively. Highest occurrence of AMR *E. coli* was observed in environmental, human and rodent samples. Week wise occurrence revealed an increased in AMR between 7^{th} to 13^{th} week in host related samples and kids had highest (P> 0.05) AMR as compared to does. Rodent samples had significantly higher (P> 0.05) AMR followed by human toilet swab and soil among the environmental samples throughout the study period. A total of 913 *E. coli* isolates were screened for presence of genes encoding AMR and it was observed that majority of the isolates carried *tetA*, *qnr*B (62.87%), *sul2* gene (53.01%), *qnr*S (30.66%), bla_{CTX-M} (30.34%), bla_{TEM} (27.71%) genes and 12.69 per cent of the isolates were colistin resistant. Occurrence of TRG, FRG and SRG was consistent in host related and environmental samples, however a significantly (P> 0.05) higher occurrence of ESBL genes was evident in human, soil and rodent samples. A cyclical pattern in occurrence of AMR genes could be observed throughout with peak occurrence during 4^{th}-13^{th} week and all the genes revealed a decreasing trend at the end of the study. Comparison between does and kids indicated an increase and decrease in AMR genes during the study period indicating the role of antibiotic administration and host immune response. This longitudinal study is the first of its kind in India and the results of this study have provided baseline data on antimicrobial use, dynamics of phenotypic and genotypic resistance to antibiotics in *E. coli* in intensive goat production system.

Annexures

IX. ANNEXURE

SURVEY QUESTIONNAIRE FOR FARMS

[1] Identification of sample households

1. State/U. T:	5. Hamlet name:
2. District:	6. ward/block:
3. Town:	7. Name of the head of household:
4. Village name:	8. Age of the HH:

[2] Household characteristics

1. Household size:	9. leased – out:
2. Household type:	10. total possessed land (7+8+9)
3. Religion:	11. land cultivate:
4. Social group:	12. land irrigated:
5. whether own any land:	13.cooking:
6. if yes in item 5, type of land owned: homestead only	14.lighting:
7. owned:	15. Income of the HH:
8. leased – in:	16. Sources of Income:

2. ***Household type***: [1] agriculture, [2] non-agriculture, [3] regular wage/salary earning, [4] casual labor in agriculture, [5] casual labor in non-agriculture.

3. ***Religion***: [1] Hindu, [2] Islam, [3] Christianity, [4] Sikhism, [5] Jainism, [6] Buddhism, [7] others

4. ***Social group***: [1] Scheduled Tribe, [2] Scheduled Caste, [3] Other Backward Classes, [4] others

13. ***Primary source of energy for cooking***: [1] firewood and chips, [2] LPG, [3] Gobar gas, [4] Dung cake, [5] Charcoal, [6] kerosene, [7] electricity, [8] others.

14. ***Primary source of energy for lighting***: [1] kerosene, [2] other oil, [3] candle, [4] gas, [5] electricity, [6] others.

Section B: - Farm characterization

17. What is the size of your farm:

18. How many sheds do you have on this farm:

19. What is the approximate number of Goats in each shed:

20. Type of breed:

21. What is the total number of animals on this farm

22. What is the approximate size of the each shed

23. Reason for goat rearing

[1] Income generating [2] self-consumption [3] Both

24. Production activity

[1] Breeding [2] Fattening [3] Both

25. Feeding Mechanism

[1] Stall feeding [2] Grazing [3] Both

26. Main feed for goats

[1] Green [2] Concentrate [3] Mix of both

[4] Silage [5] Any others

27. Breeding practices

[1] Natural [2] Artificial Insemination

Section C: - Health and Hygiene

28. Hygiene and Disease Control Practices

a. What precautions do you and others in your farm take when entering Goat shed?

[1] No precautions [2] Gloves [3] Mask [4] Foot dips/rubs [5] others ----------

b. System of rearing practiced

[1] All in all out [2] Replacement as and when needed

c. How do you clean the Goat sheds?

Frequency of Cleaning sheds:

4. Do you vaccinate the goats? If yes, please list, to the best your knowledge all diseases for which they have been vaccinated:

Vaccine	Month

30. Do you isolate or quarantine Goat? [1] Yes [2] No

32. What are the manifestation/s that tells you the pigs are sick:

33. How do you treat such illness:

34. Do you have any formal training on the diagnosis of disease in animals?

[1] Yes [2] No

Section D: - Use of Antibiotics in Goat Production

35. Do you give your Goat any antibiotics? [1] Yes [2] No

36. Source of concentrate feed:

37. Are you aware of antibiotics (AGPs) included in the feed? [1] Yes [2] No

38. Do you keep records of drugs used on your farm? [1] Yes [2] No

39. Who usually administers all these drugs to the goats?

[1] Myself [2] Farm Manager (if different from self) [3] My farm worker

[4] A veterinarian [6] Para-Veterinarian [7] Other (specify) _____

40. Do you usually consultant anybody the use of antibiotics? [1] Yes [2] No

41. Where do you purchase the antibiotics used in your Goats? (Click all that apply)

[1] Veterinary Pharmacy [2] Human Pharmacy [3] Individual veterinarian

[4] Supplied by contractor (for contract farmers)

[5] Other specify _____ [6] Don't known

42. When was the last time that a round of antibiotics was given to one of your pigs?

Condition	Antibiotics Used		Duration
	Name	Dosage & Route	

For what purpose were the antibiotics given?

[1] Growth promotion [2] Disease treatment [3] 1&2

[4] Prevention of disease [5] Not sure

43. How far is your farm from the following locations?

	Km		Km
Veterinary pharmacy/Chemist		Human health pharmacy/chemist	
Veterinary clinic/hospital		Human health clinic/hospital	
Market		Major town	

46. Are you member of livestock – related Community – Based Organization (CBO)/Self Help Groups? [1]Yes [2] No

Section D: - Knowledge and Perceptions of Antibiotic use

47. Answer Yes/No

a. Is antibiotic necessary to prevent infection Yes/ No

b. Antibiotics are helpful in improving growth rate Yes/No

c. Use of Local herbs/therapy is useful than antibiotics to treat sick animals Yes/No

48. Is Goat rearing causing any harm to children/household? Yes/ No

49. How do you dispose manure of the farm:

50. How do you dispose the dead kids/adults if any in the farm:

51. What is the average mortality in the farm:

52. Do you have any Pest control measures in the farm: